

Chernenko, Hart and Reagan

The first primaries in the US election campaign could yet help unlock the log-jam on arms control. President Reagan now has an incentive to move quickly for an agreement with the Soviet Union.

WHAT possible connection can there be between what happened last week in Moscow and New Hampshire, the New England state which has so far outwitted rivals seeking to steal the distinction of being the first to hold primary election in presidential election year in the United States? In Moscow, Mr Konstantin Chernenko, the new Soviet leader, responded to President Reagan's earlier signals that an East-West accommodation would be welcome with a list of steps that might be taken on arms control. In New Hampshire, registered Democrats in what is traditionally and by temperament a Republican state surprised the pollsters (and perhaps even themselves) by voting for Senator Gary Hart as the Democratic Party's candidate for the presidential election in November. Their decision was echoed at the weekend by the Democrats of Maine. And the consequence of that, by a simple piece of political arithmetic, is that President Reagan will now have to respond positively to what Mr Chernenko had to say.

The calculation is straightforward. When, a month ago, Mr Reagan declared himself a candidate for a second term as president of the United States, his chief rival seemed to be Mr Walter Mondale, President Jimmy Carter's vice-president. Given the electoral advantages of an incumbent president, Mr Mondale must then have seemed a comfortable opponent. Hart's wins in New Hampshire and in Maine will have undermined that sense of security, if only by reminding the President and his advisers that in the coming election, as when President Carter was elected in 1976, the voters of the United States may decline to follow the agenda their party leaders have written for them. Whether or not Hart's campaign prospers, the choice of the Democratic candidate will now be postponed, perhaps until the convention planned at San Francisco in July, while the competition between Hart and Mondale, each of whom is committed to some version of a freeze on nuclear weapons, will increase the importance of arms control as an election issue. So, since there will not be much time between July and November and because Mr Chernenko is unlikely to want to help Mr Reagan to be re-elected, the administration in Washington has every reason to move quickly towards an accommodation.

Test ban

What can it do? The simplest but also the best immediate course is to take up Mr Chernenko's proposal that the threshold test-ban treaty, signed in 1974, should promptly be ratified. The treaty requires that the signatories should not test nuclear weapons yielding more than 150 kilotons of TNT equivalent and also provides that, once the treaty has been ratified, each side will have to give the other details of its testing sites and their seismic characteristics and information about weapons tests carried out and that there should be at least two calibrating underground explosions at each testing site. Such data would help enormously to avoid uncertainties about remote verification of the kind that only a few weeks ago provoked the US Administration into accusations that the Soviet Union has been cheating. Indeed, the benefits of ratification are so obvious and immediate that it is hard to understand why the administration has not long since jumped upon them. Now, with the electoral wind blowing the way it is, both the administration and the Republican Senate would seem to have little choice.

Ratifying this single treaty will not, however, keep the electoral

wolves from President Reagan's door nor seem to Mr Chernenko to be an adequate response to what he had to say last week. Ratifying the treaty, signed bilaterally in 1976, on the conduct of peaceful nuclear explosions would similarly fall short of what is required, especially now that even Soviet interest in this clumsy technique for making holes in the ground has diminished, but the agreement that on-site inspection should be allowed under the terms of the treaty should nevertheless be a useful precedent for the future. But as things have turned out, in New Hampshire and in Maine, what the President needs is an agreement that will seem to US electors to be a much more substantial constraint on the development of nuclear weapons.

There are only two fields in which there has been anything like enough preparation for an agreement to be within reach — the comprehensive test-ban agreement (within an ace of signature in 1980) and some extension of the Salt II agreement on strategic arms based on the past two years of negotiations at Geneva, now in suspended animation. The second is the better bet, for a comprehensive test-ban would require that the British Government should be involved (probably no great difficulty) and would in present circumstances be incomplete without the adherence of France and China (quite a different matter). So the US Administration has no choice but to put aside the scepticism of arms control agreements that has informed the past three years, to put forward the draft of a treaty on the reduction of strategic arms, to plead for a resumption of the Geneva talks (in April?) and to hope for an agreement before November.

If the administration feels coerced by events it should take comfort in the knowledge that some such step will in any case be needed by this time next year if the next review conference of the Non-Proliferation Treaty, due in August, is not to be yet another shouting match with the non-nuclear powers. Five years ago, the non-nuclear signatories of that treaty left the nuclear powers in no doubt that more substantial progress was required of them. On the present showing, they have nothing to report except that they have failed to agree on what should be done. Unless something can be said just over a year from now, the treaty may fall apart.

The administration's underlying difficulty, chiefly of its own making, is however more serious. The best part of four years in office seems to have persuaded the Reagan Administration of two awkward doctrines — that bilateral agreements with the Soviet Union on arms control are politically undesirable, militarily dangerous and probably unattainable on equitable terms, but that public support for the strategic doctrines by means of which the deployment of nuclear weapons has been legitimized has now melted away. This, it now emerges, is why the administration is pinning so much of its hopes on star wars, the ambition to make defences against hostile missiles so reliable that neither diplomatic agreements nor the deployment of strategic nuclear weapons would be necessary. The obvious snag is that the feasibility of star wars defences could not possibly be demonstrated for at least a decade, and is unlikely even then to satisfy legitimate military needs. Meanwhile, the United States, its allies and their opponents will somehow have to look to their security, of which arms control has become an indispensable component. President Reagan should have understood this from the beginning of his term of office, and may yet regret that he has had to wait for Mr Hart's early successes at the polls to drive the lesson home. □



Engineering innovation

Improved engineering education is the key to the new industrial revolution.

OPTIMISM, a rare commodity during the long recession of the past ten years, seems to be taking hold again among the governments of the industrialized world, and in a way that promises that the economic doldrums may in due course end for good. For after decades of lip-service to the belief that science and technology are necessary (but not sufficient) conditions for prosperity, governments seem now to be behaving as if the truth were self-evident. What else can explain the unexpected decision last week of the ten members of the European Community that funds would be found to launch the Esprit programme of collaborative research in information technology? Or the renewed commitment of the United States Government to federal support for basic research, reaffirmed by the 14 per cent increase asked for in the budget for the coming financial year, beginning on 1 October? In these cheerful circumstances, the outstanding problem is how to translate good intentions into practice.

Two problems stand out, how to spend public money on the support of industrial innovation and how to find people who will use it imaginatively but wisely. It is important that governments now setting out to bring about another industrial revolution should first learn what can be learned from the often depressing experience of recent years of which there is, unfortunately, a surfeit.

Pride of place should go to education and to engineering education. Circumstances are much changed since the heyday of the nineteenth century's industrial revolution. The engineers on whom the burden of that upheaval fell, people like Watt (a few decades earlier) and the Stephensons in Britain and Edison in the United States, were in no sense academic but, rather, practical men whose ingenuity centred on the intuitive development of new machines. Tempting though it may now be to hope that people (women as well as men) of the same temperament might conjure endless prosperity from technical innovation, that is no longer possible. Wider access to higher education in most industrialized societies has ensured that all but a tiny fraction of creative engineers are launched on their careers from academic institutions. There is, however, good reason to suppose that the old hankering after empirical ways persists among academic engineers. Research in the standard academic pattern is less common and less successful than in basic science, but academic engineers (faculty members and students alike) have traditionally made important contributions to the solution of practical industrial problems.

This is one reason why, in the United States, the National Science Foundation deserves applause and support for its plan to create industrial centres in conjunction with university engineering departments, looking to industry not merely for equipment normally beyond the reach of academic institutions but for practical problems to solve. For what it is worth, institutions of this kind are not new; in Britain in the past fifteen years, a private foundation (the Wolfson Foundation) has made grants to support more than a hundred academically based but industrially oriented centres of this kind, many of which have been remarkably successful. The National Science Foundation seems to be planning to build industrially oriented centres that will be individually larger, and whose role in the education of engineering students will be more deliberate. This could yet be the best way of meeting the crying need that engineering graduates should acquire further skills before going out into the cruel world — and perhaps even of tempting their teachers to stay longer in an academic setting. Certainly the experiment, to be mounted later this year, deserves a fair wind.

Elsewhere, as in Britain, there are more serious problems to tackle. The Engineering Council (see p.103) seems to share with others who have taken a view of engineering education the view that universities have failed the industrial community, forgetting that at least in the British environment the failure until recently

has been that of the industrial companies which consistently undervalued the engineers whom they have employed. Even if the Engineering Council now has grounds for thinking that attitudes have changed, no useful purpose would be served by having the University Grants Committee prescribe the proportions of university budgets to be spent on engineering education. A more legitimate demand would be that universities should so organize their affairs, in the years ahead, that they can respond flexibly if, as seems likely, the demand for engineering education should increase; as things are, too many British universities have responded to the troubles of the past few years by making over-rigid arrangements to preserve the present balance of student numbers in different fields. And the Engineering Council should not forget that, in present circumstances, engineering education is not the only fruitful source of industrial innovators.

The question of how to spend money on innovation, not innovators, is more difficult. Unhappy experience suggests only one firm rule — that neither politicians nor their civil servants can be trusted with decisions of this kind, for they are prone to use public funds for the rescue of dying industries, not for the creation of the new. Similarly, but less certainly, there are dangers in the attractive device of collaborative research designed to provide a common basis of knowledge and skill on which commercial companies can then build new machines. The popular legend that much of the Japanese success in recent years stems from such programmes is overstated, and takes too little account of the meagre financial contribution of the central government. Elsewhere, the most common difficulty is that of devising a programme of collaborative research that is neither so exclusively concerned with general principles as to seem academic nor so pointed that it benefits chiefly those who happen to carry to work. Could this be part of the reason why Admiral Bobby Inman's collaborative venture on behalf of the United States computer industry is so slow off the ground (see p.99)? It will be interesting to see whether the managers of the Esprit programme launched last week in Brussels are more successful. □

Space station trouble

NASA in the United States has only itself to blame that its plan to build a space station is in trouble.

REPORTS that the United States is about to build a space station are, so to speak, exaggerated. That seems to be the burden of the intelligent criticism by the congressional Office of Technology Assessment of the plan being canvassed by NASA on behalf of the US administration to design a habitable platform somewhere in orbit about the Earth (page 101).

The issue is quite simple. NASA considers that there should be a space station, but is for the time being unable to say exactly (or even roughly) what it would be for, except perhaps as a kind of talisman. The hope that two years of further work will clarify the issue is almost certain to be disappointed, for what will by then have emerged is that there are several possible uses for a space station, each of which would predicate a different kind of platform in a different kind of orbit.

As things are, however, some proven needs stand out. One is that there should be means of recovering satellites from geosynchronous orbits about the Earth. Such satellites may each cost several hundreds of millions of dollars and their lifetimes could with great economic advantage be extended by the replacement of relatively minor components. Another is a durable space platform which would be a splendid base from which to mount scientific observations. For the time being, there is no economic use for in orbit materials processing.

Putting a space station in a geosynchronous orbit would however be exceedingly expensive, and would not even be the most economic way of recovering satellites in the same orbit. It follows that the practical obstacle to efficient use for a space station is uncertainty about the kind of vehicle that would make the journey from what would presumably be an orbit nearer the Earth. So why not develop that first. □

Thermonuclear fusion

US community asks for next machine

Washington

FUSION researchers in the United States are making a strong pitch for a new tokamak machine that will reach plasma ignition — the point at which fusion becomes self-sustaining without additional external heating. To maintain the “three-year lead” gained by the start-up of the Tokamak Fusion Test Reactor (TFTR) at Princeton, Dr Harold Furth, director of the Plasma Fusion Laboratory there, told a congressional committee last week that the United States should start serious planning now for the new machine so that construction could begin in 1987. Furth said that the new machine, dubbed the Tokamak Fusion Core Experiment (FTCX), could be operational by 1992.

The existing TFTR is expected to reach scientific break-even, the point where the fusion reaction is generating as much power as is being applied to heat and confine the plasma. The new TFCX would boost confinement (the product of density and confinement time) by roughly an order of magnitude over that which TFTR will achieve pushing the plasma to ignition with confinement times of as long as 5 minutes. The cost of TFCX, for which no detailed

more than the inflation rate, and there seems little likelihood of much change. The budget is now \$471 million; \$483 million has been requested for fiscal year 1985.

Although TFCX has received broad support from the fusion research community — both the Energy Research Advisory Board and the Magnetic Fusion Advisory Committee (MFAC) have called it the number one priority in fusion research — a go-ahead for the project may cause some strains. Several witnesses at last week's hearing expressed concern about what would happen to the base of fusion research at the universities if TFCX were to be built within the present budget. Ronald Davidson, director of the Massachusetts Institute of Technology's Plasma Fusion Center and chairman of MFAC, asked for a gradual “ramp-up” by 20–40 per cent to

allow TFCX to begin without harming the existing programme. The Department of Energy is expected to allocate \$10–20 million in fiscal year 1985 for conceptual design studies, although the budget contains no specific request for the project. The first indication of how the fusion research budget will be treated in the light of TFCX will come next year, when money for actual design work may be requested.

Congress is seen as generally supportive of the fusion effort — particularly because of the threat of foreign competition. Dr Allan Mense of McDonald Douglas Company, representing the Institute of Electrical and Electronics Engineers, ritually invoked the Japanese and Soviet menaces at last week's hearings, noting that while the US fusion budget has grown by less than 5 per cent per annum since 1977, Japan's programme has been growing at 30 per cent per annum. The closest competitors to TFTR are the Joint European Torus in the United Kingdom and two machines expected to begin operating in late 1985 or early 1986, Japan's JT-60 and the Soviet Union's T-15.

Stephen Budiansky

US electronics

Fifth generation stillborn?

Los Angeles

A CONSORTIUM of US electronics companies created to build the “next generation” of computers before the Japanese is having difficulty attracting first-rate scientists to join the effort.

“Assembling the talent and getting the research under way has not been going as fast as I would like” says Bobby Inman, president of the Micro-electronics and Computer Technology Corporation (MCC), now setting up shop in Austin, Texas.

Mr Inman, former US Navy admiral, director of the National Security Agency and deputy director of the Central Intelligence Agency, said he recently offered key managerial jobs to four people who turned him down.

A quarter of the electrical engineering and computer science faculty at the University of California at Berkeley has been approached by MCC and none has shown interest. Leading scientists at the Massachusetts Institute of Technology and Carnegie Mellon University, who attract talented subordinates, have also refused offers.

MCC was created last year to pool the resources of 14 high-technology companies for a crash programme to develop advanced “fifth generation” computers using so-called expert systems and artificial intelligence programs. Copying a similar Japanese effort, MCC will share scientific breakthroughs, if any, with its member companies. Details of how that is to be done have still not been worked out and the consortium remains controversial.

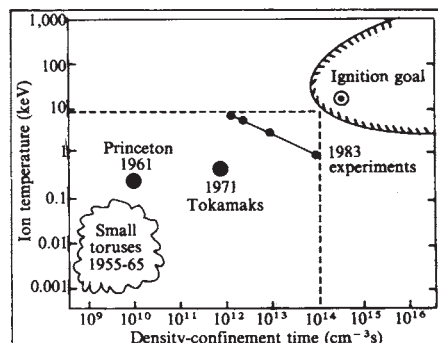
MCC is an unproven enterprise, according to scientists who have refused Mr Inman's offers. There is a shortage of people able to design and program advanced computers and the best of them are content with their present jobs. MCC is trying to establish a “quality research team” in a hurry but, the scientists say, such a thing cannot be rushed.

Some academics said they do not feel an ex-military officer can create the “optimum environment for research”, although Mr Inman's Washington connections are invaluable for MCC.

Several states sought to have MCC established on their turf, expecting to accrue prestige and scientific talent. But states that lost seem to be faring better than Texas, which won. Stung by criticism that he bungled efforts to land MCC, the governor of California this year substantially raised faculty salaries. Georgia, MCC's second choice, used publicity gained from the competition to lure new high-technology companies to Atlanta. North Carolina did likewise. The governor of Texas, meanwhile, is saddled with a promise to bring MCC \$23.5 million in private donations. This is on top of \$40 million from state coffers, which have been depleted by falling oil revenues.

Mr Inman is not perturbed. “It's awfully early for a report card”, he says. “I'm a good five months ahead of my worst case and about four months behind my fastest track.” Of an expected 300 employees, “We have 64 people here as of today. The cast of characters continues to surge.”

Sandra Blakeslee



Break-even conditions for thermonuclear fusion based on the “Lawson criterion”. The diagonal line labelled 1983 experiments shows the results of various machines reaching the ignition conditions independently (although not all at the same time).

design has yet been produced, is estimated at less than \$1,000 million.

Furth said that recent results have given the fusion research community new confidence that ignition can be achieved. Separate experiments around the country have independently demonstrated the conditions necessary for ignition; ion temperatures of the order of 100 million degrees, confinement times of the order of a second, and density-confinement — time factors of $10^{14} \text{ cm}^{-3} \text{ sec}$.

The Department of Energy's magnetic fusion budget has suffered from fairly tight constraints in recent years, growing at no

European research

Community agrees on Esprit

Brussels

COULD research bring Europe together again? It almost seemed so in Brussels last week, where an extraordinary meeting of research ministers saw Britain not only giving the go-ahead for the Esprit programme for research in information technology but actually suggesting an increase in its budget.

At the Athens summit last December, which ended in chaos, Britain and West Germany had blocked the start-up of Esprit pending agreement on much wider financial issues — including Britain's "subscription" to the Community and the matter of vast agricultural surpluses. Since December, West Germany has indicated that it would lift its objections to the programme but the British position showed no signs of moving.

That was changed last week by the British information technology minister, Mr Kenneth Baker, who agreed that Esprit should go ahead with a Commission commitment of 750 accounting units (AU) over five years (approximately 1 AU = US\$1), 50 million more than had been asked for by the Commission, much to its officials' surprise. The Commission's con-



Commissioner Etienne Davignon

tribution is to be backed unit for unit by European industry — making a total budget of 1,500 million AU (£868 million).

Baker enthused about "an important day for Europe, an important day for collaboration". This was "the best day for European research for a long time", said French research minister and council president Laurent Fabius.

So why did Britain hesitate at Athens? According to Conservative Member of the European Parliament, Mr Robert Moreland, the Conservatives (who form the British Government) had always been firmly behind Esprit, and the programme had been temporarily blocked only as a protest at the failure of Athens.

According to M. Fabius, the European Community has lost two million jobs because it has fallen behind the United States and Japan. Esprit is part of the plan to get these jobs back. Mr Baker is "absolutely convinced" that the information technologies will be the main motive

force for the economy in the next 20 years and pointed to the investment of £1,500 million in related British industries in the past four years.

Even so, total spending on Esprit is dwarfed by that of at least two US companies. Officials here see Esprit more as a psychological aid to Europe's pre-competitive research. The programme will concentrate on getting the best responses to calls to tender for projects in just five key sectors (see below) but there will also be discretionary funds for high-risk innovative work in fields such as artificial intelligence.

Restoring European confidence is the objective of another project suggested by Vicomte Etienne Davignon, the Commissioner for science and research, over the ministers' lunch — a European video-communications network to link the ten member governments with Community institutions. It will be, according to M. Fabius, "a world first, symbolic of European attainments".

Esprit itself has sprung in just over three years from an idea tossed around by a few European civil servants to round table discussions to define industrial needs in October 1981, last year's £7 million pilot projects and, now, a fully-fledged programme spanning the decade to 1994.

The programme is thus one of what M. Fabius called the three pillars of European

research. The others are the so-called stimulation programme aimed at creating greater mobility and multidisciplinary collaboration and the biotechnology programme. After an experimental phase which got under way last June, the council is now looking for more scientific exchanges through laboratory twinning and by means of multinational centres of excellence, while biotechnology is regarded as an important engine of European economies in twenty years' time — as well as a way of turning agricultural surpluses into industrial raw materials. Further decisions on both these programmes are expected later this year, perhaps before the French presidency runs out at the end of June.

Last week's council made little progress on other programmes on its agenda such as basic technological research, radiation protection and non-nuclear energy. But some £4 million extra was found for work on acid rain and toxic substances.

Further ahead, there is likely to be a debate about priorities for European research. The Commission has committed itself to "budgetary stringency" on Esprit, implying that no extra research money will be called for, so that other research programmes will come under closer scrutiny. The European Commission says that there is no question of making economies elsewhere, but member states will be anxious to settle priorities for Community research so as to settle the research budget between now and 1988.

David J. Price

What will Esprit achieve?

ESPRIT is a radical step for the European Community. The only other on a comparable scale is the nuclear fusion programme, but that does not promise quick returns.

The details of the five-year programme will be redefined each year. So each year there could be surprises. One already in the 1984 programme just defined is the inclusion of an effort in optoelectronics, until recently considered far-fetched.

The principal objectives of the 1984 rolling programme, which demands international cooperation on projects and divides research for management purposes into microelectronics, software, advanced information processing, office systems and computer integrated manufacture, are:

- To give Europe a substantial place in the microchip market, by producing (by 1988) "a soundly-based industrial 1 micron capability in a number of sites" with substantial progress towards sub-micron capability (that is, very high density chips) by that date.
- Optoelectronics, aiming at an "optical system on a chip" within 5 years.
- To integrate natural language interfaces with expert systems within four to seven years. Dialogue with such systems should be able to offer "cooperation

and understanding of personal behaviour. . .".

- The production by year five or six of "full-scale commercially valuable" software that is able to "learn" from experience.
- In the study of interfaces, to be in a position by year five to start design of a system to recognize handwriting; and the modelling of human aural and visual perception.
- The development of an operational "advanced workstation", including voice recognition and speech synthesis, for the office within five years.
- The development of wideband optical local area networks.
- The development of flexible machining systems and robotics, including a generalized robot language.
- The establishment of centres of expertise, continuing education and research in computer-integrated manufacture.

Eager Commission officials had already issued informal requests for tenders for the Esprit projects before the favourable research council decision on Esprit (see above), so the programme should be up and running by this summer.

Robert Walgate

US space station

Alternatives to space agency plan

Washington

THE US National Aeronautics and Space Administration (NASA) has not yet won its long-running campaign to be allowed to build a space station. Although President Reagan gave the project his approval in January, NASA must still persuade Congress to vote money for it. And in recent hearings on Capitol Hill, Congress's Office of Technology Assessment (OTA) has emerged as a strong critic of NASA's approach.

Officially, OTA declines to say whether it supports a space station or not, but its director, Dr John Gibbons, reminded the Senate Budget Committee last week that the President had approved plans for "a" space station, not "the" space station. While NASA wants to build a major facility costing \$8,000 million, a forthcoming OTA study is expected to argue that many of the same objectives could be achieved for a fraction of the cost by adopting a slightly more modest design.

Urging Congress to consider the detailed requirements for a space station before approving NASA's plans, Gibbons sketched out three other possibilities.

- A low cost option could comprise an orbiter capable of supporting up to six people for three weeks. It would be paired with two modestly-powered unmanned free-flying platforms, one in polar orbit and the other in low-inclination orbit. Both could be leased from the private sector and tended periodically by orbiter crews. There would also be a new orbital manoeuvring vehicle that could move between the orbiter and free flyers at other altitudes.

- A "relatively modest" option would be similar in most ways but the lifetime of the orbiter would be supplemented by a permanent orbiting facility with wide-band communications and up to 15 kilowatts of power. Unlike the low-cost option, this would allow long-term experiments using human beings as subjects or requiring human attendance — the kind of experiments of particular interest to the life and materials sciences. Although much cheaper than the more elaborate designs suggested by NASA, this option would give the United States "substantially greater" opportunities than those provided by the Soviet Union's Salyut programme.

- A higher cost option would add a reusable orbital transfer vehicle designed to move back and forth between its service module in low Earth orbit and geostationary orbit. That would enable it to service and modify spacecraft in geostationary orbit and could lay the basis for more efficient travel between the Earth, the Moon and the planets.

Gibbons stressed that OTA was not recommending any of these options, but had developed them to make the point that there is a wide range of possible space

station designs and that US space capabilities could be usefully enhanced by spending much less than the \$8,000 million envisaged by NASA. OTA has developed a more extensive list of options and their associated costs (see table).

OTA's criticism of NASA's approach is based on a long-standing belief that US

civilian space policy has lost its sense of direction and that a sensible decision on whether to build the kind of space station NASA wants cannot be taken until overall space goals have been settled — preferably by a national commission. In the closest OTA has come to direct opposition to NASA's plans, Gibbons told the budget committee that neither NASA nor the government as a whole had tried to develop a national space agenda that could justify the new project.

Peter David

OTA's comparative space station designs

	Date available	Cost (\$1,000 million)	Power (kW)	Pressurized volume (cubic feet)	Crew size	Time in orbit	Comments
Shuttle orbiter	Now	None	7	2,000	6	10 days	Can accept Spacelab
Extended duration orbiter	1990	0.4	20	5,000	5	50 days	Limited laboratory space
Spacelab as permanent structure	1990	2	6	5,000	3	Unlimited	Limited crew
NASA plans initial	1992	8	90	6,000	8	Unlimited	Orbital manoeuvring vehicle plus two free flying platforms
NASA plans mature	1996-2000	20	200	10,000	20	Unlimited	Reusable orbital transfer vehicle plus several platforms

Polish environment

Immediate action imperative

POLAND is facing a possible ecological catastrophe, according to Dr Antoni Kuklinski, head of the Committee of Spatial Development of the Polish Academy of Sciences. In the government newspaper *Rzeczpospolita*, he identified three especially "weak links" which had reached "breaking point" — the natural environment, the technical infrastructure (transport, power supplies and water management) and the poor conditions in large urban areas.

The situation could be improved, he said, only if industrial investment were cut to 30 per cent of the national total. At the same time, the housing sector must learn to make better use of the available means. The whole structure of investment must be changed, with greater powers given to local "self-government" bodies. Poland, he said, must invest in its environment lest the shortage of fresh air become worse than the shortage of shoes (a notorious problem).

Dr Kuklinski's warning is not the first to emerge recently in the Polish media. Last August, Warsaw Radio noted that in the upper Silesian industrial belt, 430 per 100,000 inhabitants died prematurely because of environmental conditions and that the death rate was 50 per cent above the national average. Circulatory diseases were 15 per cent more frequent, cancer 30 per cent and pulmonary diseases 50 per cent more frequent. Infant mortality was 13 per cent higher. One doctor recommended that Silesia should be treated as a closed industrial zone in which people should

work for not more than 20 years. Those not working should live elsewhere.

This view is reinforced by a report which embodies the findings of a seminar held in Lublin last autumn on the chemical threat to the Polish environment. This notes that, according to a survey carried out in 1982 by the Planning Commission of the Council of Ministers, 27,000 km² of Polish territory, encompassing a population of 11 million (more than 30 per cent of the total population), is under severe ecological threat. Special attention is given to the classification of air pollution in terms of its sulphur dioxide toxicity equivalent, since recent international alerts on acid rain have made Polish planners aware of the sulphur dioxide hazard, if not of other pollutants. According to these figures, the hazard from heavy metal dust from the metallurgical and engineering industries is equivalent to 13.8 million tonnes of sulphur dioxide a year, while gases from the chemical industry (other than sulphur dioxide and carbon monoxide) have a sulphur dioxide equivalent of 1.26 million tonnes. The annual emission of sulphur dioxide by Polish industry is 2.45 million tonnes.

The report stresses that the whole pollution problem was worked out anew in the 1982 "Theses" for the reform of the economy, and that action on three "strategic" problems (food supplies, housing and restoration of the ecological balance) will be introduced during the 1986-90 plan.

Vera Rich

Ocean research

Japan to hit bottom

Tokyo

A FRANCO-Japanese deep-sea exploration programme using France's brand-new undersea research vessel, the SM97, is to begin in Japanese waters this June. Final plans for the two-year project — named *Kaiko* (Trench) — were announced by the University of Tokyo's Ocean Research Institute (ORI) this week after the Ministry of Finance agreed to provide Y 800 million (\$0.35 million) as Japan's half-share of the costs.

The areas to be explored in the Japan Trench and Nankai Trough — where the Pacific and Philippine plates collide with the Eurasian plate upon which Japan stands — are of more than academic significance for Japan. Deep seismic activity in the subduction zone is the major trigger for the shallower earthquakes which threaten Tokyo and other parts of Japan's densely populated eastern seaboard.

Nine years of negotiations between ORI and France's Centre National pour l'Exploitation des Océans (CNEXO), within the framework of an older general agreement on oceanographic cooperation, have already gone into the project. Early plans to use the French bathyscaphe *Archimède* were abandoned when the French decided it was too old. Instead, the go-ahead was given for the SM97 which, with a depth limit of 6,000 metres, can reach the bottom in 97 per cent of the ocean areas.

Construction of the SM97 is now nearly complete and, after testing in the Atlantic, its maiden research voyage will be in Japanese waters. Weather permitting, some 60–70 dives will be made in the spring and summer of 1985, in seven regions off the Japanese coast. As well as the Japan Trench and the Nankai Trough, surveys will be made of the triple junction of the Eurasian, Philippine and Pacific plates; the Suruga-Sagami Trench complex, where the northernmost boundary of the Philippine plate is colliding with the Japanese landmass; and the first Kagoshima Sea Mount. This remarkable undersea mountain, first discovered in 1980, returned to prominence this week with the announcement of the results of a high-definition sonar survey by Japan's maritime safety agency which confirm the movement of the Pacific plate. The undersea mountain, the size of Mount Fujiyama, has split into two as the past few hundred thousand years of plate movement have carried it westward over the edge of the Japan Trench. One-half of the mountain has slid 1,700 metres down into the trench and is separated by nearly five kilometres from the rest of the mountain, poised on the edge of the trench.

This summer, before the SM97 arrives, the French oceanographic vessel *Jean Charcot* will spend two months making a detailed map of the bottom topography of

the regions using Seabeam, probably supplemented by photography from the unmanned towed deep sea vessel *Epaulard*. The SM97 carries a crew of three — two scientists and one operator — down to 6,000 metres and is equipped with video-cameras and a manipulator which can be used to take cameras' samples from the ocean bottom. Equal numbers of French and Japanese scientists will man the vessel

and its mothership, *Nadir*, and representatives from the United States and the United Kingdom have also been invited to participate. Results will be used to choose the best areas for the SM97 dives. One key scientific problem for the project, says Noriyuki Nasu, director of ORI, is to discover why the subduction in the deep Japan Trench proceeds rapidly with the formation of only a small accretionary wedge, while in the shallower Nankai Trough, subduction is slow and a large accretionary wedge has developed.

Alun Anderson

Dioxin exposure

US Air Force reassures veterans

Washington

A US Air Force study* has found scant evidence for veterans' claims that their health was damaged by herbicides sprayed by US forces in Vietnam. The Air Force called the results of the five-year epidemiological study "reassuring" for the veterans and their families. But the argument will continue, most conspicuously in the federal courts.

Thousands of Vietnam veterans claim to have suffered cancer, birth defects in their children and other medical problems as a result of their exposure to the 19 million gallons of herbicides the Air Force used during the war in "Operation Ranch Hand", which aimed to destroy crops and deny cover to Vietcong forces. Residues of dioxin were present in all of the herbicides; Agent Orange, of which 11 million gallons were sprayed, contained 2 p.p.m. dioxin.

The Air Force study compared the "Ranch Handlers" with a control group drawn from airmen who flew cargo missions in Vietnam and who were not involved in herbicide spraying. There were significant differences between the two groups in the incidence of certain birth defects, skin cancer and in "miscellaneous" liver disorders. But there appeared to be no consistent pattern of the frequency of these maladies in relation to the doses of dioxin that different groups of Ranch Handlers were calculated to have received. For example, the number of birth defects in the children of enlisted men increased with exposure to dioxin, whereas officers with the highest calculated exposure to dioxin reported the fewest birth defects. For skin cancer and liver disorders, no correlation with dose was found. Moreover, there was no evidence of the health problems specifically associated with dioxin exposure. None had soft-tissue sarcomas or chloracne.

The report suggested some grounds for suspicion of the data on birth defects and skin cancer. The birth defect results were based on self-reporting by the veterans and have not yet been confirmed by medical records; the skin cancer data were not adjusted for possible differences in sunlight exposure between the Ranch

Handlers and the control group.

Robert Taylor, a Washington attorney representing some 1,000 veterans who are suing Dow Chemical and other manufacturers of the herbicides, claims that the report showed that "science is catching up" with what the veterans have known for some time — that Agent Orange has indeed injured their health. He questioned the validity of the exposure estimates used in the report, which are calculated by dividing the total amount of dioxin-containing herbicide used during an airman's tour of duty in Vietnam by the number of personnel who performed that airman's same task during the same period. "Nobody really knows who was exposed and to how much", he said.

The study does not include some two million ground troops who may have also been exposed to the herbicides. The Air Force maintains that ground troops would have received a tiny fraction of the dose that its personnel received from the herbicide sprays blowing back into aircraft, from leaking hoses and during manual cleaning of the spray tanks. According to the Air Force, 1,269 of its troops were involved in Operation Ranch Hand between 1962 and 1971. Ninety-seven per cent of them filled out a questionnaire for the study, and 87 per cent agreed to undergo a physical examination, which was conducted by civilian physicians on a blind basis.

The veterans' lawsuit, which has been certified as a class action open to all 2.5 million Vietnam veterans, is scheduled to go to trial on 7 May in US District Court in Brooklyn, New York. Nearly 16,000 veterans have joined the suit, which alleges that the chemical companies knew of dioxin's dangers but did not inform the government. The chemical companies are arguing that the government was equally aware of the hazard but took no steps to protect its troops, and that the companies were merely complying with government purchase orders.

Stephen Budiansky

*An Epidemiologic Investigation of Health Effects in Air Force Personnel Following Exposure to Herbicides. Baseline Morbidity Study Results, USAF School of Aerospace Medicine, Brooks Air Force Base, Texas; 24 February 1984. Available from: Surgeon General, US Air Force, Washington, DC 20314.

UK engineering education

More demands on universities

ENGINEERS on both sides of the Atlantic seem intent on getting a better deal for their profession. In the United States, one major battle has already been won now that the National Science Foundation has been convinced of the need to spend \$10 million a year on a network of university-based engineering centres to encourage interdisciplinary research and to bring academic engineers closer to industry (see below).

In Britain, the Engineering Council, the new and increasingly outspoken statutory body with responsibility for professional education and certification, wants more radical measures. The council this week announced that it wants to see an extra £40 million a year spent on engineering courses in universities and polytechnics, with a further £200 million in incentives to industrial companies to provide training places.

The council hopes for a 10 per cent swing in student numbers from arts-based to science-based subjects within the decade, with most of the extra going to engineering. (Some 15 per cent of British university students are studying engineering of some kind.) But, as engineering students cost twice as much to educate as arts students, the problem of money rears its ugly head. The council argues that shortages of trained engineers are constraining many British companies with interests in high tech-

nology, so that no price is too high. Its latest pronouncement is very much a political gesture: its goals will be out of reach unless the British Government is persuaded to put more resources into higher education than the omens suggest is likely. It is widely feared in the universities that a cut in government support by one per cent or more each year is now in prospect.

Many in the universities would accept this part of the argument, but the council has also risked losing academic friends by coming as close as it dared to accusing the universities of mismanagement of their funds. Sir Kenneth Corfield, the council's chairman, said that universities had to often used their recurrent grant to maintain the *status quo* rather than "in the way that was intended".

That view is not new to university vice-chancellors, but it is the first time it has been stated so publicly. At least for the next five years, Sir Kenneth wants a proportion

of the universities' recurrent grant to be earmarked for engineering by the University Grants Committee, presumably to stop the same thing happening in future. Universities, however, jealously guard their right to spend their grant as they wish. Mr Maurice Shock, vice-chancellor of the University of Leicester, said last week that he is "entirely opposed to the notion that a body can sit in London and dispose of the thousand and one considerations of individual universities".

The Engineering Council also wants more academics to take their sabbaticals in industry, and estimates that £7 million in earmarked funds is needed for the purpose. That idea is likely to be more acceptable to the universities and, as greater collaboration between universities and industry has been one of the most popular choruses of recent years, it might make some headway. The Committee of Vice-Chancellors and Principals has within the past month set up a committee to investigate the obstacles to collaboration; the transferability of pensions is one area likely to be scrutinized.

Tim Beardsley

US engineering education

Tripartite centres mooted

Washington

THE Reagan Administration seems to be pinning a great deal of hope for engineering education on a plan to establish engineering centres in close association with university engineering departments. The National Science Foundation (NSF)'s draft budget for the next financial year includes a request for £10 million to launch the scheme, which has been applauded by a panel of the National Academy of Engineering. Meanwhile, NSF is working on the details of the scheme, when university departments will be invited to submit proposals.

Creation of the centres was proposed last year by NSF's engineering directorate in a working paper that argued that while much attention had been paid to the falling number of engineers prepared to work in universities, relatively little had focused on the quality of research produced by university engineers. In too many areas, it complained, industrial applications had outstripped the fundamental knowledge developed at universities. To keep pace, universities needed to foster research across the traditional sub-disciplines, and nurture closer contacts with large-scale engineering projects undertaken in industry.

In a report last month, the National Academy of Engineering endorsed the NSF scheme — with a vengeance. While conceding that NSF might have to begin by establishing fewer than ten centres in the first year, the academy wants NSF to aim eventually for a network of 25 centres, at an overall cost of at least £100 million. Engineering departments that want to join

the new scheme should ensure that at least 10 per cent of their postgraduate engineering students and at least three full-time members of staff are involved in the centre.

Despite its enthusiasm, the academy has warned NSF that the centres should be seen as only one of several steps urgently needed to raise the standards of engineering research and education. Equally important are measures to lure talented engineers into university careers, an increase in the size of NSF research grants to engineers and replacement of outdated equipment. The academy also insists that the new emphasis on interdisciplinary research should not be allowed to impoverish fundamental disciplinary research.

NSF's handling of the new centres will be closely watched by an engineering community already critical of the foundation. Many university engineers maintain that NSF has paid insufficient attention to engineering, and a bill has been introduced in Congress to turn NSF into a National Science and Engineering Foundation. The bill would increase the number of engineering directorates at NSF and call on the foundation to sponsor applied research not yet ripe for industrial exploitation.

Dr Edward Knapp, NSF's director, has meanwhile been emphasizing the importance the foundation already places on engineering. In a recent speech at Duke University in North Carolina, he pointed out that half of the awards granted last year under NSF's new "young investigator" scheme were earmarked for engineers, and he promised that the same proportion would be designated under the expanded scheme in 1985.

Peter David

Hall back in favour

BEIJING'S "Hall of Science" — a complex of more than 30 assembly and conference halls which was closed down during China's Cultural Revolution — was formally reopened last month.

The complex, established in 1963 under the patronage of Premier Zhou Enlai, at one time played a major role in the scientific life of the capital. The Red Guards, however, had condemned it on the grounds that it was a "Petofi club" — a term of abuse drawn from the "Petofi circles" of intellectuals in Hungary before the 1956 uprising.

Beijing scientists have been pressing for some time for the reopening of the Hall of Science. Last month's reinauguration was formally stated to have come about as a result of top-level party and state "attention" to the problem. Accordingly, Zhou Peiyuan, chairman of the China Science and Technology Association, reminded the 800 "elated" scientists at the reopening ceremony that their return to the complex was intended to help them "to accelerate development in the various branches of science, to pursue major scientific and technological subjects in the course of national economic construction and to serve the development of socialist material and spiritual civilization".

Vera Rich

Social sciences

Lobby success in Congress

Washington

LEGEND has it that Congress's scientific committees possess a document known as the "silly research book", a continuously updated collection of explanations and defences of social science research supported by the National Science Foundation (NSF). The book is brought to budget debates to help blunt the criticisms of the many congressmen who, like Wisconsin's William Proxmire, believe the bulk of social science to be little more than a cruel hoax perpetrated on the taxpayer by the intellectually self-indulgent. When the Reagan Administration arrived in office, even the silly research book seemed to offer little hope of averting a nemesis for social science research. The administration lost little time in making plain its antipathy towards social science: in proposals for NSF's budget for fiscal year 1982, the White House asked for reductions of 75 per cent in the social and economic sciences, 67 per cent in the behavioural and cognitive sciences and 39 per cent in the anthropology programme.

Four years later, all that has changed. Social science continues to prosper, if not thrive, within NSF and the other federal agencies which sponsor research. In the administration's budget request for fiscal year 1985, spending for social science by NSF is almost back to its 1980 levels.

Why the change of heart? At least part of the reason is the unexpected success of the US social science community in using traditional congressional lobbying techniques to build up support. At the heart of this lobbying effort is a fledgling Washington-based organization called the Consortium of Social Science Associations (COSSA), established in 1981 by half a dozen learned societies representing key social research disciplines. Led by a combative historian, Dr Roberta Balstad Miller, COSSA is given much of the credit for pulling off a remarkable reversal of social science's fortunes.

Although it represents a collection of learned societies, COSSA operates much like any other political lobbying organization in Washington. Last year its spokesmen testified in front of a dozen congressional committees to argue the case for sustained government support of social research. The high-water mark for COSSA came in a fraught budget debate in July 1981, when social scientists were still stunned by the administration's proposal to cut NSF's social research by 75 per cent. Before the debate, which was to determine whether Congress would accept the administration plans, COSSA launched an unprecedented campaign to muster political influence.

Some 4,000 social scientists were asked by their disciplinary associations to call on their local congressman and urge opposi-

tion to the cuts. Every member of Congress received a letter from COSSA and many were visited by delegations of academics from their home states. Sympathetic congressmen were even provided with texts of arguments that could be made on the floor of the House of Representatives to support the case against the administration. In the end, the administration's budget for NSF was defeated by 264 votes to 152, after a debate studded with unaccustomed allusions to the national importance of social science research.

At the very least, the debate appeared to teach the White House's Office of Management and Budget (OMB) that there could be no politically painless cuts in social science research. And the creation of

COSSA provided social science for the first time with a single-minded watchdog that can be relied on to howl in outrage whenever the disciplines it represents are treated unfairly in the budget process.

Yet social science has not been entirely unchanged by the advent of the Reagan Administration. In many agencies, support for social research has changed to reflect short-term policy goals with clear links to the agency's mission. Some years ago a famous (and, by all accounts, excellent) study of the population of nineteenth-century Philadelphia was given substantial funds by the National Institute of Mental Health. It is doubtful whether a similar project would be supported today. COSSA may have been able to blunt much traditional antipathy towards social sciences, but the Reagan Administration has also seen to it that there are fewer candidates for the silly research book.

Peter David

Europe's environment

Glimmer of hope for forests

EUROPEAN environment ministers last week accepted the principle of reducing air pollution from industrial plant by means of fixed emission limits. Spurred by concern about damage to forests in central Europe, the ministers adopted a framework directive that will allow tough emission controls to be introduced in later legislation. One major implementing directive, on limiting emissions from large combustion plants, is already waiting for formal decision.

The controversial clause in the directive adopted last week says that fixed emission limits shall be introduced only with the unanimous agreement of the Council of Ministers. That represents a compromise wording insisted upon by Britain, which is opposed to the use of Community-wide emission limits if they can be avoided. An earlier draft of the framework directive allowed for the introduction of fixed emission limits by a two-thirds majority, a feature which Britain contested. The revised clause also allows ministers to take account of "excessive cost" and the "nature, quantity and harmfulness of the emissions".

The British view is that emission limits laid down in Brussels would introduce an unnecessary tier of authority. Britain's chief industrial air pollution inspector, Dr Leslie Reed, recently argued forcefully that the British concept of "best practicable means" might be adequate to deal with many pollutants. But new British legislation will now be necessary. The implementing directive on emissions from large combustion plants that is now awaiting formal discussion incorporates a belt-and-braces approach: fixed emission limits for new industrial plant, and also large percentage reductions in total national emissions of sulphur dioxide, nitrogen oxides and dust. It is left to individual countries' discretion how compliance should be achieved.

The compromise now reached would in

theory allow any member state to veto the further directives, although it seems unlikely that any country would risk the odium of all the others on such a sensitive issue. Scandinavian countries were this week making representations to Britain to reduce sulphur dioxide emissions. Officials in Britain will admit privately that there is a good case for taking some action to reduce sulphur dioxide emissions, but are not impressed by the Community's argument that uniform emission standards applying to all new plant would lead to fairer economic competition.

The real argument is now likely to begin in earnest, over the numbers to be plugged into the implementing directives. The Central Electricity Generating Board has condemned the present targets of 60 per cent reductions in national sulphur dioxide emissions and 40 per cent reductions in nitrogen oxide and dust emissions as excessive. Even a 50 per cent reduction in its own emissions would, it says, cost £1,500 million in capital investment and £300 million a year, raising electricity prices by up to 15 per cent. The board also argues that there is no good evidence that reducing emissions would achieve the desired effects. Many others think that the balance of the evidence has now swung in the favour of reducing emissions. Much is made of a study by the US National Academy of Science which, according to one directive, "established . . . the existence of a linear relationship between emissions and the quantity of acid depositions".

Before adopting any detailed directives on measures to combat air pollution, the council of ministers will want to seek an opinion from the European Parliament. A rapporteur has already been appointed to prepare comments on the directive relating to combustion plants.

Tim Beardsley

Plant genetics

Safeguarding the pool

A BINDING international commitment to the conservation of plant genetic resources and to setting up an international commission to monitor work in this field are embodied in an agreement proposed in the recent report of the 22nd session of the Food and Agricultural Organization (FAO). However, the form of agreement, yet to be ratified by member governments, leaves unanswered many questions about administrative and financial relationships with existing organizations. Moreover, by confining the proposed commission to FAO members, the Soviet Union and East Germany, both important holders of plant genetic resources, are excluded as is the United Nations Educational, Scientific and Cultural Organization, which under the Man and Biosphere programme is the main UN agency responsible for *in situ* conservation. The United States, Japan, Canada and Switzerland are thought to have expressed opposition to the agreement and France, West Germany, the Netherlands and the United Kingdom have objected on procedural grounds, because last minute changes to the proposed composition of the commission, instigated by Mexico, prevented adequate consultation between delegates and their home bases.

Governments that ratify the agreement will declare their readiness to "ensure that plant genetic resources of agricultural interest will be explored, preserved, evaluated and made available for plant breeding for the benefit of all human beings". The resources include cultivars, related wild or weedy species and special genetic stocks. The proposed agreement would result in a coordinated network of national, regional and international centres, including base collections which together would form an "international gene bank", under the auspices or jurisdiction of FAO.

Accessions must be held in more than one institution because conservation efforts are subject to human, social and economic failure. For example, of 1,000 sugar clones held in Papua New Guinea in 1955, only 204 remained in 1975. And the fate of the Ethiopian wheat institute is at present unknown. The scale of the problem is immense; there are already 293,000 accessions of cereal crops held in the Soviet Union, United States and Philippines alone. The Asian Vegetable Research Station in Taiwan has acquired 10,000 soybeans in six years. And the International Rubber Research and Development Board, in its annual report published last week, estimates that there are 200,000 rubber genotypes kept as seeds in its Germplasm Centre in Manaus, Brazil. The pressure to collect and store plant genetic resources is increasing as the destruction of wild habitats, natural disasters, urbaniza-

tion and commercialization of agriculture all combine to threaten *in situ* conservation. For example, massive loss of Afghanistan's native wheats occurred in the 1970s after drought, harvest failures and catastrophic famine were followed by importation of thousands of tonnes of seed corn.

FAO fears that commercial pressures contribute to genetic erosion and non-availability of germ plasm, because the adoption of high-yielding strains causes abandonment of genetically rich primitive stocks, and private companies hold large gene banks. However, the existence of a plant breeding industry depends on an adequate return on capital investment protected by plant varieties rights legislation. In addition, developing countries are increasingly acquiring their own breeding and seed-producing facilities and material is freely available from existing international institutes, such as, for example, the Wellsbourne vegetable collection partly supported by Oxfam, the largest UK overseas charity.

The FAO plan may eventually duplicate, replace or absorb the Consultative Group on International Agricultural Research (CGIAR) and the International Board for Plant Genetic Resources (IBPGR). CGIAR supports a wide programme, including the 12 international agricultural research centres, partly supported by countries in which they are situated. It also supports IBPGR, which has effectively pursued a large range of activities including providing basic equipment for national gene banks in 20 developing countries, sponsored 250 collecting missions in more

Eastern AIDS

THE first confirmed cases of acquired immune deficiency syndrome (AIDS) in the socialist bloc were reported last week from Czechoslovakia, where the victims are said to be a Slovak and an African. Hitherto, although there have been unconfirmed rumours of cases in East Germany, the official attitude has been that AIDS, like herpes, is a disease of capitalism.

In Poland, however, the threat of AIDS was taken more seriously, since neglect of the hospital service during the Gierek era has led to cramped conditions and frequent cross-infection due to re-use of disposable syringes and catheters. Last autumn, the weekly *Polityka* assured the public that if the disease reached Poland, the health service would be able to cope.

Following the reports from Czechoslovakia, the Polish Institute of Hygiene has issued a special leaflet to help doctors make a swift diagnosis, and blood donor stations are to take steps to ensure that people with AIDS do not give blood. According to a spokesman for the Ministry of Health, no cases of AIDS have been identified in Poland. Many doctors fear, however, that AIDS symptoms may already have appeared but have been mistaken for some other disease.

Vera Rich

than 70 countries, supported training programmes and published directories on plant genetic resources. However, these autonomous, philanthropic, non-profit organizations lack "legal personality", and in face of the increasing politicization of the issues surrounding plant genetic resources, FAO seeks to create a more institutionalized structure and forum for discussion.

Sarah Tooze

Nature index of biotechnology stocks

12-Month high	12-Month low	Company	Close previous month	Close 28 Feb.	Change
23 1/4	9 3/4	Biogen (Switzerland)	12 1/2	13	+1/2
6 1/4	1 3/8	Bio-Logicals (Canada)	2	1 1/2	-1/2
16 1/8	7 1/4	Bio-Response (USA)	11 3/4	10 3/4	-1
19	10 1/4	Cetus (USA)	13 1/8	11 1/4	-17/8
15 1/2	6 1/8	Collaborative Research (USA)	8 3/4	7 5/8	-3/8
39 7/8	15	Damon (USA)	17 3/4	17 1/2	-1/4
34 1/4	14 3/4	Enzo-Biochem (USA)	23	16 3/4	-6 1/4
18 7/8	6 3/4	Flow General (USA)	8 3/4	7 3/4	-1
49 3/4	25 7/8	Genentech (USA)	37 1/4	33 1/2	-3 3/4
17 3/4	6 1/2	Genetic Systems (USA)	8 3/8	6 7/8	-1 1/2
23 1/4	12 1/2	Genex (USA)	13 1/2	13	-1/2
31	14 1/2	Hybritech (USA)	21	14 3/4	-6 1/4
22 1/4	10	Molecular Genetics (USA)	14 1/2	13 1/4	-1 1/4
23 1/4	10 1/2	Monoclonal Antibodies (USA)	12 1/2	11	-1 1/2
73 1/2	42	Novo Industri A/S (Denmark)	55 3/8	52 1/4	-3 1/8
30 1/4	13 3/8	Pharmacia (Sweden)	18 7/8	16 3/4	-2 1/8

For over-the-counter stocks, bid price is quoted; for stocks on the American and New York exchanges, the transaction price. *Nature's* weighted index of biotechnology stocks stood at 163 on 28 February, compared with 181 a month earlier. Data from E.F. Hutton, Inc.

Animals do have rights

SIR — I believe your leading article, "What rights for animals?" (*Nature* 306, 522; 1984) contains three important fallacies. First, implicit in the article is the assumption that we do at present put a sufficiently high value on the human right to life and freedom from suffering. While children are still allowed to die of hunger because affluent nations prefer to convert vegetable foods to meat (with around 90 per cent wastage) rather than undertake the difficult task of achieving fair distribution of essentials, this cannot be so.

Second, the article initially claims that retarded humans have rights, while animals do not, because ill-treatment of subnormal humans may lead to similar treatment of humans who are "different" but not "inferior", while ill-treating animals cannot have this effect (because of the clear distinction between animals and humans). It then goes on to say that "wanton" ill-treatment of animals (that is, cruelty for fun) is wrong because it corrupts the human values of compassion. However, if this is true, one must accept that some degree of continuity exists between the emotions which govern our behaviour towards human beings and those which occur when we interact with animals. In fact there is a considerable body of evidence which suggests that cruelty towards animals and violence towards humans do have common causes.

If compassion towards animals is not a qualitatively different emotion from compassion towards humans, and since a very great deal remains to be done to remove the injustices which many humans suffer, it can only be a healthy sign that there are people whose "personal compassion outweighs any desire to eat meat". Thus animals could well be judged to have rights on the basis of the arguments which your writer accepts as valid in the case of retarded humans. (Although I do not myself accept that such humans have rights only because being cruel to them might lead to mistreatment of "real" people.)

Third, I think the article assigns an unbalanced degree of importance to certain human states of mind. Clearly there is a moral difference between inflicting suffering for fun and inflicting equivalent suffering as an unwanted by-product of some desired end. However, where that end is itself trivial, I suggest that the subjective feelings of the human agents involved are of rather less moment than those of the animals which actually experience pain or distress. Furthermore, this emphasis upon motivation is dangerous because there is unfortunately much evidence that very similar reasoning is frequently employed in situations where people are hurt. Industrialists who would not dream of physically abusing a child see nothing wrong in promoting bottle-feeding to mothers who lack facilities to use this

method safely; babies die as a result, but the manufacturers feel no guilt because they were "only doing the best they could for their shareholders".

Finally, may I address your censure that animal rightists lack a sense of humour. I do not believe that those responsible for decisions which affect animals fully appreciate what very great distress is caused to many quite ordinary people by the infliction of suffering upon animals. Whether we like it or not, very large numbers of people do view their pets as members of the family, and react accordingly when similar animals are harmed. It is as unreasonable to expect them to keep, for example, the Draize test of shampoos, in proportion as it would be to expect an assault upon one child to be kept in proportion. When such people repeatedly hear eminent scientists defending experiments which clearly are trivial, or saying that it is impossible to draw a line between cosmetic and medical experiments it is hardly surprising if they conclude that all animal experiments would prove equally unnecessary if closely investigated.

ROSEMARY RODD

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SIR — The leading article on rights for animals contains several flaws. For example, you state that "there are simply no consistent or universal principles that imbue animals with 'rights' as exercised by humans". However, there is considerable controversy and disagreement among contemporary philosophers about the proper analysis of the notion of rights, human or animal. There is also heated disagreement about the proper substantive grounds for granting, or not granting, recognition of moral rights even to humans. Your justification for defending the rights of severely handicapped humans is purely consequentialist (once we curtail the rights of one group of humans, it will not be long before we curtail the rights of others). It could, therefore, be seen as a denial that such human beings have their own intrinsic moral rights.

One cannot, therefore, decide the issue of how animals should be considered merely by asserting that humans have moral rights that are different. We must, instead, squarely face the challenge laid down by several modern philosophers as to why humans and animals are treated so differently. In this regard, it is important to provide an adequate answer to why we should treat mentally incapacitated humans (who are apparently not rational nor self-aware) any differently from vertebrate animals which may be self-aware and capable of reason. Crook has recently discussed the concept of animal consciousness and has indicated that considerable moral implications stem from whatever

conclusions are reached (J.H. Crook, *Nature* 303, 11-14; 1983).

It should also be noted that human recognition of "a moral obligation to treat animals with compassion and to respect their undeniable interests" is, at best, inconsistent and spotty. The pet dog and cat receive considerably more protection than the pig in a farrowing crate and yet surely pigs, dogs and cats have very similar mental capacities. The only reason that we can keep pigs in a manner not fit for a dog is because our interest in pigs is different from the interest in dogs. (In some societies, pigs are kept as pets and dogs eaten.) Our "respect" for the interests of the animals is primarily a concern for our own interests in the animal's well-being.

Obviously, we have to draw lines in establishing some form of moral construct for humans and the rest of creation. It seems intuitively obvious that bacteria do not and should not have the same moral status as ourselves but neither are they without any value whatsoever. However, it is equally obvious that we have not thought carefully about the place of mammals in the moral continuum and that there are many inconsistencies in our attitude towards and treatment of our fellow vertebrates.

ANDREW N. ROWAN

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Israel squeezed in

SIR — I was a little surprised to read in *Nature* (2 February, p.408) that Israeli scientists will not be able to participate in the 7th International Biotechnology Symposium. On the contrary, as the result of discussions and negotiations undertaken by the International Council of Scientific Union's Standing Committee on the Free Circulation of Scientists, arrangements were made for Israeli scientists to participate. Your correspondent received on 6 January a letter from the organizers advising him that his visa could be obtained on arrival. He also received a cable on 27 January informing him that Indian missions in Cairo, London and Rome had received cables giving instructions to clear his visa on application. It is easy to understand the concern of a scientist when having problems in obtaining a visa but to call for a boycott of the symposium when the person concerned knew that delicate negotiations were proceeding with the Indian authorities not only shows a singular disregard of the problems involved but is also unlikely to facilitate future negotiations, especially if the visa problem concerns the person involved.

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No new ways of treating AIDS

The discovery of what may be the agent responsible for the simian analogue of AIDS does not, for the time being, help with understanding the human disease or simplify its management.

San Francisco

ONE way and another, this splendid city has the dubious reputation of being the AIDS capital of the world. Although new cases now arise more frequently elsewhere, in New York for example, this is where the unusual occurrence of Kaposi's sarcoma and lung infection by the organism *Pneumocystis carinii* were first recognized to be symptoms of an underlying profound defect of the immune system now known as acquired immune deficiency disease, or AIDS. What follows is an account of a brief conversation with some of the staff at the clinic set up at the San Francisco General Hospital for the treatment of patients with the disease.

The first thing to say is how little there is to say. The second, that in the circumstances it is remarkable that the staff of the clinic (mostly physicians in their thirties) should so quickly have cultivated such an admirable blend of detachment and understanding in the face of the frustration which is their daily lot.

Even the news from the Davis campus of the University of California that Dr Preston L. Marx's group there has identified a B-type retrovirus as the probable causative agent of the simian (monkey) analogue of human AIDS seems to offer no relief from the frustration. That report, said to have appeared in the current issue of *Science*, does not, after all, suggest that the causative agents of simian and human AIDS are identical or even prove that the agent of human AIDS is a retrovirus. People here are keen to point out that a DNA virus with properties akin to those of hepatitis-B virus would equally explain the tendency of the human AIDS virus to go antigenically to ground.

For what it is worth, the agent of simian AIDS seems to have been about for longer than its human analogue. Although the retrovirus identified at Davis is derived from a rhesus monkey that died with immune deficiency more than a year ago (and although it has since been cultured in monkey kidney cells and also transmitted to other monkeys), Dr Marx thinks it likely that evidence for its occurrence can be found in the records of the rhesus monkey colony going back at least to 1975, perhaps even earlier. The proof that the virus is a retrovirus hangs on its shape (seen in electron micrographs) and on the presence of reverse transcriptase (for converting RNA into the appropriate DNA) in tissue cells which harbour it. The difficulty, for

physicians, is that even if the agent responsible for human AIDS may be similar in kind, there is no obvious way in which this can be demonstrated quickly and no obvious way, thereafter, to the development of prophylactic techniques.

So how gloomy should one be about human AIDS? The physicians note that the treatment they are at present able to provide for their patients in the clinic amounts to nothing but the treatment of the adventitious symptoms of the underlying condition. And even there, the prospects for intervention are poor. Drugs that could be counted on to succeed in the treatment of, say, *Pneumocystis carinii* infection in healthy patients turn out to be toxic in patients with AIDS. So it may be necessary to treat a patient suffering from such an infection with two drugs consecutively in the knowledge that this will exhaust the repertoire of what is available, meaning that a second bout of infection may be intractable.

This is why immediate hopes centre on the trials about to be mounted in San Francisco of the efficacy of genetically engineered γ -interferon and of interleukin-2. Both studies should be under way within six weeks. There is also a study of the usefulness of α -interferon among people suffering from blood-cell disorders that may be precursors of outright AIDS but which may also be less severe consequences of infection by the same causative agent.

What, in the circumstances, can patients suffering from AIDS be told? The simple answer seems to be the truth. The physicians point out that the population of male homosexuals most at risk is also, in San Francisco, an intelligent population of people able and willing to assume responsibility for their own fate. Patients therefore get to know that if they are properly diagnosed as suffering from AIDS, the prognosis is gloomy. Many take the view that the best they can do is to arrange that the quality of their life shall be as far as possible preserved. Hospitalization is wherever possible avoided.

So how serious is the outbreak of human AIDS? And is it fair to describe it as an epidemic? While the number of cases so far recognized is still only just over 400, people are quick to point out that the incidence of the disease is high among the population at risk, perhaps several hundred a year per 100,000 single men. Moreover, there seems no sign of a decline in the rate at which

cases accumulate in the city, which appears to have increased month by month except for a brief hiatus in mid-1983. Sooner or later, of course, the rate at which new cases occur should level off, but when that will be can be told only when more known of the natural history of the disease and of how the infection is acquired.

Epidemic is, then, the right word; that is what the physicians say. Indeed, part of their anxiety is that the population at risk may be lulled into a false sense of security by optimistic reports that some new development may spell the understanding and imminent treatment of the disease.

Indeed, they have a still more chilling vision of where the present epidemic may lead. The agent of AIDS, whatever it is, seems to be in its virulent form a genuinely novel infection of human beings. Since the condition was first recognized for what it is, there has naturally been a diligent search of past medical records for evidence of the characteristic adventitious symptoms, among which Kaposi's sarcoma should be the most distinctive. So far, the search has been unsuccessful. So what, in 1979, when the first deaths occurred from what was two years later recognized as AIDS, can have happened to the causative agent? The analogy with the agent of syphilis, a skin disease before it became a venereal disease, shows that infectious agents can radically change their habit and the nature of the disease they cause. May, then, the agent of AIDS change again, becoming in the process a more generalized infection of people?

The opinion that the AIDS agent may be an infectious agent in the course of adaptation to a new ecological relationship with human beings is supported by evidence as well as by imagination. The difference between the symptoms manifest among male homosexuals and among the others groups among whom the disease occurs, emigrants from Zaire or Haiti, for example, suggests some such variability.

Others working in the field give a different but no less chilling account of what is happening. Noting the prevalence of lymphadenopathy among the population at risk which appears not always to become frank AIDS, they imply that the infectious agent may already be widespread in the human population, in which case the objective of research should be not so much to identify the virus as to tell what trauma makes infection become so damaging.

John Maddox

Immunology

The T-lymphocyte antigen receptor — elusive no more

from Alan F. Williams

THE molecular nature of the T-lymphocyte antigen receptor has been the most mysterious and controversial issue in immunology over the past decade. One major question has been the extent to which the receptor is related in structure to immunoglobulins. Three papers¹⁻³, from two independent groups, in this issue of *Nature* (pages 145, 149 and 153) are the first to provide molecular evidence that the answer, in brief, is that at least one of the two chains of the receptor looks rather like an immunoglobulin light chain (see Fig. 1).

B and T lymphocytes are quite distinct cell types. B lymphocytes differentiate in the bone marrow and their only known function is to respond to an antigen and produce cells that secrete antibody. T lymphocytes differentiate in the thymus and interact directly with other cells to regulate the immune response or to kill cells expressing the appropriate antigen. B cells can simply bind foreign antigen, but most T cells can only see antigen presented at the surface of another cell where it is recognized along with molecules encoded by the major histocompatibility complex (MHC). This dual recognition could involve one receptor for each determinant or the more favoured possibility of one receptor recog-

nizing both foreign antigen and MHC antigen^{4,5}. Clearly molecular data are needed to resolve these issues.

Although it has long been known that the antigen receptors of B lymphocytes are cell-bound immunoglobulins⁶, the many claims that T lymphocytes express immunoglobulin gene products have lacked clear molecular evidence. Studies using gene probes finally convinced most workers that immunoglobulin genes are not expressed in T cells and at the end of 1982 it was concluded that nothing was known about the molecular nature of the T-cell receptor⁷.

This situation began to change in 1983 with two reports of monoclonal antibodies that were specific for individual T-cell clones or T-hybridoma lines and which inhibited T-cell functions^{8,9}. In both cases the antibodies bound to a structure that was a disulphide-linked dimer of polypeptides of about 40,000 and 43,000 molecular weight (mouse) and 43,000 and 49,000 molecular weight (human). These structures are similar to a glycoprotein dimer identified as a tumour-specific antigen of a mouse T lymphoma¹⁰. Peptide maps showed that the two chains in the dimer were distinct and also that the

equivalent chains from clones of different specificities had some peptides that were different and possibly others that were similar or identical⁸. So far there are no published sequence data on these polypeptide chains.

The authors of the papers in this issue took a different approach. Their strategy was to clone cDNA copied from T-cell mRNA, select out the clones that were specific for T lymphocytes and finally to search among these clones for any that might potentially encode part of the T-cell receptor.

The surprise is that in both the mouse³ and human¹ studies, equivalent molecules have been identified. Sequence similarities are at the level of about 80 per cent in the carboxy-terminal halves of the molecules which suggests that the human and mouse forms of the same gene have been identified. Both sequences contain one segment that can be convincingly aligned with an immunoglobulin V domain and another that fits well with an immunoglobulin C domain. The model in Fig. 1 would fit either molecule with the exception that the human molecule is predicted to have two N-linked carbohydrate structures rather than five as shown for the mouse molecule.

The evidence that the molecules identified are part of the T receptor is strongest for the mouse molecule whose genes have been shown to be rearranged in T cells but not in other cell types². Also a region of varying sequence associated with the constant region has been established³. Thus it seems clear that a gene set has been discovered which is similar to, but distinct from, those of the immunoglobulin light chains. If the second chain identified in the monoclonal antibody studies has a similar structure, then the T receptor will look much like a MHC class II antigen or one Fab fragment of an immunoglobulin (Fig. 1).

However this may be far from the complete story. Hedrick *et al.* describe cDNA clone 86T1 in most detail but they also present partial sequences on three other cDNA clones². All appear to have the same carboxy terminus but their sequences differ at the amino terminus. The level of sequence diversity seems to be much

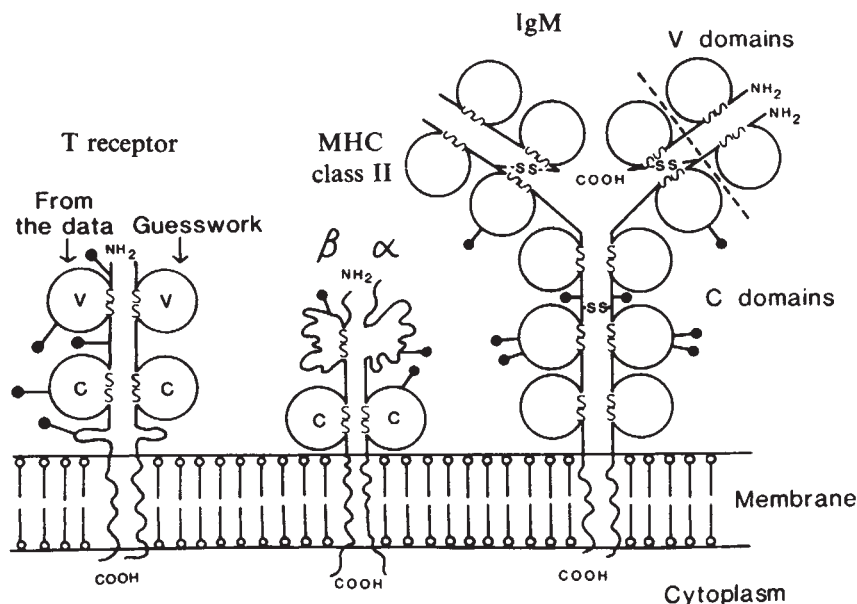


Fig. 1 Model for the mouse polypeptide 86T1 plus a second chain with hypothetical domain structure, making up a T-receptor structure consistent with the monoclonal antibody data⁸⁻¹⁰, and compared with a class II histocompatibility antigen and IgM structure. Circles marked V are like an immunoglobulin V domain; those marked C are more like an immunoglobulin C domain. Also shown are intrachain disulphide bonds (S), interchain disulphide bonds (S-S), and N-linked carbohydrate structures (↑). For the 86T1 molecule the positions of carbohydrate and disulphide bonds are postulated from the sequence. The interchain disulphide bonds between the T-receptor polypeptides are not shown since their positions are unknown.

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greater than in the case of immunoglobulin V domains which show highly conserved sequences outside the hypervariable regions that make up the antigen-combining site (Fig.2)¹¹. Moreover, only clone 86T1 has a domain resembling an immunoglobulin V domain. For example, clone 86T5 has no cysteine residue which could be a candidate for homology with Cys 109 in 86T1, around which is seen the greatest identity of sequence with immunoglobulin V domains. Thus it is not at all clear that a structure like that shown in Fig. 1 will be typical for all T-receptor polypeptide chains.

From now on things will move fast. All the questions that have been answered for immunoglobulin genes and sequences will be asked of the T-receptor molecules and their expression in T-cell subsets will be eagerly investigated. With luck, cDNA clones coding for the other polypeptides of the T-cell receptor will soon be obtained. It will be vital to close the circle between structures predicted from the cDNA clones and the glycoproteins discovered with monoclonal antibodies on T-cell clones and hybridomas. This can be achieved either by sequencing the proteins or by

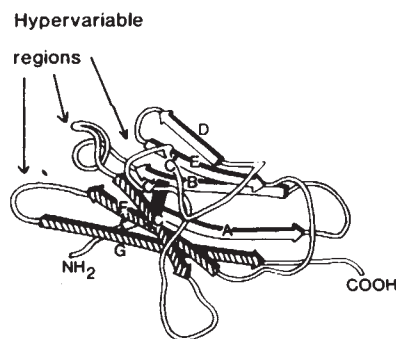


Fig. 2 Folding pattern for an immunoglobulin V domain. Antiparallel β -strands labelled A-G along the sequence fold to form two β -sheets that are held together by hydrophobic interactions and the disulphide bond indicated by the bar. The loops of sequence that form half of the combining site for antigen are labelled hypervariable regions. They show extensive sequence variation between different V domains while much of the rest of the sequence is highly conserved (adapted from ref. 14).

showing that antibodies raised against peptides predicted from the cDNA clones will react with the same structures as the

monoclonal antibodies. Hedrick *et al.* already mention that anti-peptide antibodies against their structure block T-cell responses³.

Does the structure illustrated in Fig. 1 help explain how the T-cell receptor recognizes a foreign antigen in association with a MHC antigen? Not much because the V domains presumably form a structure like the antigen-combining site of immunoglobulin which recognizes only a small area of protein or carbohydrate antigen¹². A vital point here is whether the T-cell receptor V domains will show hypervariable regions as do immunoglobulin V domains or whether, as the data of Hedrick *et al.* already suggest, they will show extensive variation throughout much of their sequence. If this is so, then the combining site of the T-cell receptor may be considerably larger than that of antibody, and this might allow a single T-cell receptor to recognize both foreign antigen and MHC antigen. □

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The tale of a falling cat

THE feline somersault shown below may not at first sight look a candidate for an historic scientific photograph, but in fact it occupies a special place both scientifically and photographically. It was recorded in 1894 on a continuous filmstrip by one of the most ingenious and productive scientific photographers of the last century, Etienne-Jules Marey, using an advanced form of his 'chronophotographic' apparatus with a rotating shutter.

On 29 October of the same year, the filmstrip was run through a zoetrope (a Victorian antecedent of the cineprojector) at a meeting of the Académie des Sciences in Paris. The falling cat sparked lively controversy among the academicians. Dropped upside down, cats virtually always manage to land feet-first, in apparent contradiction of the principle of conservation of angular momentum. The sequence of frozen images, shown below,

far from resolving the question, incited Messieurs Appell, Deprez, Fouché, Guyou, Lecornu, Lévy, Picard, West and others to debate whether the 'theorem of areas' (as the conservation law was described, following Laplace) had been photographically confounded. Indeed, Guyou — the speaker after Marey at the 29 October *séance* — started by saying that "this spontaneous turning of the animal appears impossible".

The controversy came within a whisker of becoming another case where theoreticians prove that, aerodynamically, bees cannot fly. Marey pointed out that the cat first flings its hind paws out with its forepaws retracted to rotate its head and shoulders and then reverses the procedure, extending its forepaws whilst tucking in its hind ones. On the basis of the photographic evidence, he gave short shrift to prior notions that the cat shoves itself in the

right direction at the moment of falling, in this instance pushing against the experimenter's hands; or that it somehow utilizes air resistance.

The Italian mathematician Giuseppe Peano, writing in 1895, dealt wittily with what he considered the quite irrelevant paw movements and asserted stoutly that the whole manoeuvre depended on the twisting of the cat's tail in the opposite direction to its bodily rotation. In 1969, T.A. Kane and M. P. Scher of Stanford University reopened the question with a dynamical study of the cat's fall. "The torso of the cat bends", they contend, "but does not twist". Perhaps an experiment with a tailless Manx cat would settle the issue.

Jon Darius

Selected from Beyond Vision by Jon Darius of the Science Museum (to be published in April by Oxford University Press). Reproduced by courtesy of the Science Museum, London.

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REASONS

Phase conjugate mirrors

Mirrors that reflect time

from Malcolm Gower

AFTER a decade of being a laboratory curiosity, the first practical applications are now materializing¹ of mirrors which have remarkably different properties from those used in our everyday lives. For example, light reflected from these mirrors, irrespective of their tilt, always retraces its path back to its source — sometimes with increased intensity. Even more remarkable is that transparent objects appear invisible when looked at in such a mirror, called a 'phase conjugate' mirror.

The secret is that the reflected wave is the complex conjugate of the incident wave. Thus as well as reversing the direction of light, a phase conjugate mirror also reverses its phase. Unlike a normal mirror, a diverging beam is reflected from a phase conjugate mirror as a converging beam back to its source (Fig. 1). Furthermore, if a completely transparent phase-distorting object, such as a bottle, is placed in front of the mirror, then its form, which is recognizable solely by the phase information imprinted on the transmitted wave, becomes reversed following reflection. As this reversed wave passes back through the bottle, regions of advanced phase are correspondingly retarded (and vice versa) so that the phase structure of the bottle on the emergent wave is lost, making the bottle appear invisible (Fig. 2). An observer looking into such a mirror would see absolutely nothing.

Because the wave emergent from the bottle is an exact replica of the incident wave, phase conjugate mirrors can be regarded as running the picture backwards in time for the light which falls upon them. Although

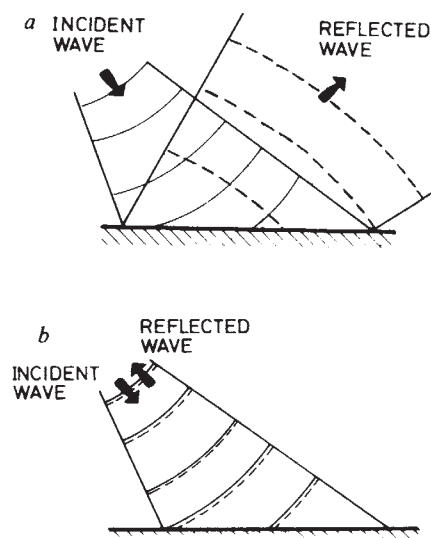


Fig. 1 Schematic diagram of reflection of a divergent light beam from a conventional (a) and a phase conjugate (b) mirror.

phase conjugation has become one of the most active areas of optics research in the past few years, many of the fundamental ideas are not new but go back to the holographic or wavefront reconstruction methods first discussed by Gabor². However, it took several years before it was realized that if a hologram made by the superposition of a reference wave and a coherent wave reflected from a subject is illuminated from the back by a reference wave in the opposite direction (conjugate) to the original reference wave, a single real image is produced which is the complex conjugate of the beam originally reflected from the subject³. With this type of geometry, it became possible to use holography to perform the phase aberration correction type of experiment shown pictorially in Fig. 2 (ref. 4). But because recording and reading of the hologram are quite separate and different stages of the process, the use of this technique in aberration correction or image projection is limited.

Only with the advent of holography in real time⁵ has the true potential of the technique been realized. In an experiment first described by Soviet scientists, a pulsed ruby laser was used simultaneously to write and read a hologram in a cell containing a dye in solution (ref. 6 and Fig. 3). Because the refractive index of the solution is modified by the local light intensity, the pattern of refractive index within the cell becomes a hologram constructed (or written) by the interference of the reference wave E_1 and the subject wave E_3 . This is then simultaneously read out by the reference wave E_2 to produce E_4 , which is the phase-conjugate of the subject wave. As far as the medium is concerned, the counter-propagating reference waves are indistinguishable, so that E_2 and E_3 can also write a (different) hologram which is read out by E_1 to produce an additional contribution to the phase conjugate wave E_4 . The result is a three-dimensional optical device (not a planar surface) which nevertheless returns the phase-conjugate of an incident wave.

This type of real-time holography is more completely and correctly described in non-linear optical terms as four-wave mixing⁷. Within this framework, when three coherent optical beams are mixed together in a medium whose refractive index changes with light intensity, it is possible to generate a fourth coherent wave whose wavelength and direction depend upon the relative angles of the beams. Waves E_1 , E_2 and E_3 may be regarded as inducing in the medium an electrical polarization which oscillates with a frequency of $\omega_4 = (\omega_1 + \omega_2 - \omega_3)$. This polarization radiates a wave at the same fre-

quency ω_4 , with a phase $\phi_4 = (\phi_1 + \phi_2 - \phi_3)$ and in the direction of the wave vector $\mathbf{k}_4 = (\mathbf{k}_1 + \mathbf{k}_2 - \mathbf{k}_3)$. For the setup depicted in Fig. 3, all three frequencies are chosen identical (degenerate) and the counter-propagating pump waves are conjugates of each other (so that $\mathbf{k}_2 = -\mathbf{k}_1$ and $\phi_2 = -\phi_1$). Thus the generated wave travels in the direction $\mathbf{k}_4 = -\mathbf{k}_3$ with a phase $\phi_4 = -\phi_3$ and so is the conjugate of E_3 .

Although real-time holography is analogous to this method of degenerate four-wave mixing (DFWM)⁸, the pictures are not completely equivalent. For example, it has been observed, and DFWM theory predicts, that it is also possible to generate phase conjugate reflections by scattering of the probe wave E_3 from a grating formed by the interaction of the pump waves with each other. This grating is uniform in space and its amplitude oscillates with a frequency of 2ω as a result of coherent oscillations in the excitation of the atoms or molecules in the medium. It has no holographic analogy.

Effects such as light-induced heating, saturable stimulated emission and absorption, molecular orientation (or the optical Kerr effect), electrostriction, surface deformation, charge migration and photo-refraction all produce changes in refractive index with light intensity. Each of these mechanisms has been used to produce phase conjugate reflections from media as

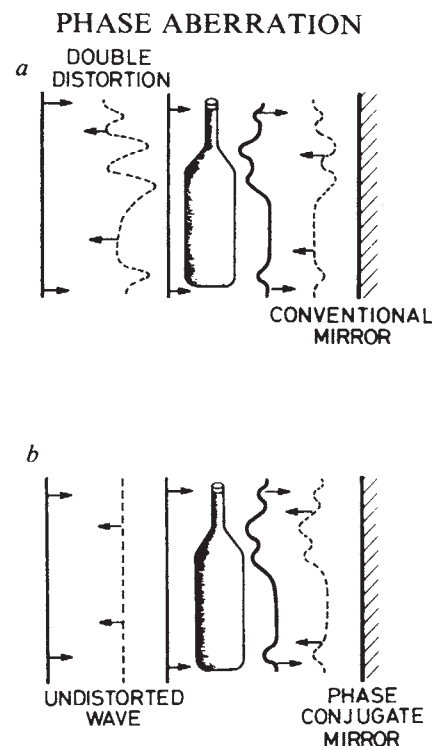


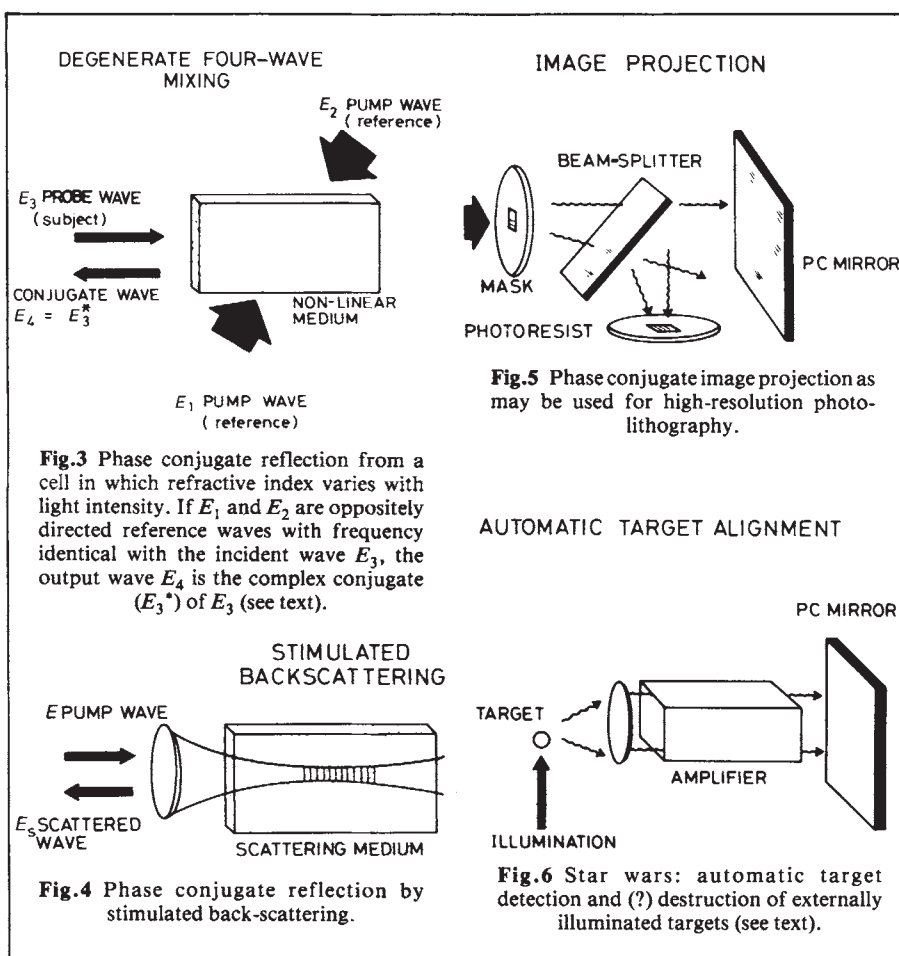
Fig. 2 Schematic diagram of correction of phase aberration by phase conjugate reflection. The phase-distribution across the wave-front is returned from the conventional mirror (a) is the sum of two refractions by the transparent bottle, but these cancel out (b) after reflection from a plane phase conjugate mirror.

diverse as solids, liquids, gases, plasmas, aerosols and liquid crystals. For some of the mechanisms, lasers with continuous powers of only a few microwatts can be used to form the mirror, while for others high-powered pulsed lasers generating millions of watts are necessary. Conjugate reflections have been observed from lasers operating in the ultraviolet to the infrared. Furthermore, conjugate image size-reduction can be produced by simultaneously reading the hologram with a shorter wavelength than that used in writing it. The magnitude of the phase-conjugate reflectivity depends upon the relative intensities of the three beams as well as on the characteristics of the non-linear mechanism which couples the light to the medium. The possibility that power can be transferred from the pump waves to the conjugate wave means that conjugate mirrors can exhibit gain, and reflectivities as high as 10,000 per cent have been observed.

There is, however, a much simpler method of producing phase conjugate reflections. Soon after the invention of the laser, it was discovered that if a high-intensity beam is simply focused into a medium, various light-scattering processes (Brillouin, Raman and Rayleigh scattering) can be stimulated to produce gain in the backward direction (Fig. 4), and that backward-travelling waves can be produced with an intensity comparable with the laser intensity. In 1972, Zel'dovich *et al.*⁹ found that stimulated Brillouin back-scattered radiation is the phase-conjugate of the (aberrated) pump laser beam. Reflectivities for the process can approach 100 per cent.

To understand this phenomenon we may regard the Brillouin wave, E_s , as being produced by the scattering of the pump light E from noise-generated sound waves which travel in the forward direction. Hence E_s is Doppler down-shifted in frequency by an amount determined by the speed of sound in the medium. Positive feedback and gain occur as more sound waves are created by the electrostrictive forces set up by the (forward-moving) pattern formed by the interference between E and E_s . The intricate coupling between the three waves leads to an exponential growth-rate for the conjugate wave which is nearly twice as great as that for all other Brillouin back-scattered waves. Hence the stimulated conjugate wave will rapidly establish itself and, by depleting the pump intensity, will further inhibit the growth of other back-scattered waves. It is almost as if the sound waves are contoured to form a moving deformable mirror which exactly matches the pump wavefront.

Within the constraints imposed by the small frequency shift of the Brillouin wave (1 part in 10^4 – 10^5), it is possible to generate almost perfect phase conjugate reflections from solid, liquids, gases and plasmas. Although this is the easiest and most popular method of making phase con-



jugate mirrors, stimulated backscattering from induced molecular vibrations (Raman), orientational (Rayleigh-wing) and temperature (thermal-Rayleigh) fluctuations have also been used. Because of their growth from noise, stimulated processes are a threshold effect and so usually require a much higher laser intensity ($> 10^9$ W cm⁻²) than needed for generating DFWM mirrors. On the other hand, stimulated Brillouin scattering does not require uniform pump waves for good conjugation, while pump beams of sufficient quality for DFWM mirrors are extremely difficult to produce for use with very high-power lasers. For these applications Brillouin mirrors are usually preferred.

The practical applications of phase conjugate mirrors are still in their infancy. Clearly they will find a use in compensating for optical aberrations of the type depicted in Fig. 2. Already they have been used to correct for aberrations due to imperfect optical elements such as windows or lenses, high-power laser amplifiers and the Earth's atmosphere. Figure 5 shows an arrangement in which a laser beam is projected through a mask pattern and a beam-splitter onto the mirror, so that an image of the mask is projected from the beam-splitter. This technique may be useful for photolithography as used in the production of silicon chips, where extremely complicated patterns are projected with high resolution over relatively large areas. Indeed, using

ultraviolet light, Levenson has achieved a resolution of greater than 500 line pairs per millimetre over an area of 0.3 cm² with such a phase conjugate lensless image projection scheme. The automatic pointing and tracking of targets can also be achieved using phase conjugate mirrors. A small glint from a target, illuminated by a low-power laser, is sent through a high-power amplifier onto a phase conjugate mirror, which sends the now very intense laser radiation back through the amplifier and onto the target to destroy it (Fig. 6).

Other applications such as mode dispersion correction in optical fibres, information processing and laser resonators with phase conjugate mirrors are all being investigated (see, for example, ref. 10). As a result of the discovery of phase conjugation with its remarkable properties, a great revival of interest in optical physics is now taking place. □

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Chemistry

Far from simple kinetics

from J. Christopher Whitehead

DESPITE the apparent simplicity of the exchange reaction $H + H_2 \rightarrow H_2 + H$, it has been a formidable task to check, experimentally, the theoretical predictions of how the energy of the reaction becomes distributed among the possible quantum states of the products and the directions in which the products recoil. Recent developments in laser technology have at least enabled two independent research groups to measure the energy of the very closely related reaction, $H + D_2 \rightarrow HD + D$.

Because it is the simplest of all possible chemical reactions involving neutral species, $H + H_2 \rightarrow H_2 + H$ has played an important part in the development of the theory of chemical kinetics. The theoretical prediction of the outcome of the reaction proceeded in two stages. First, the energy of interaction of the three hydrogen atoms involved in the reaction was evaluated for all possible geometries. As a result of the considerable computational problems involved, it was not until 1956 that a truly *ab initio* quantum mechanical calculation was completed (Boys, S.F. *et al.* *Nature* **178**, 1207) and not until 1978 that the calculations had been refined to the point where they are believed to be accurate for all configurations (Siegbahn, P. & Liu, B. *J. chem. Phys.* **68**, 2457).

The second stage of the theoretical studies used the calculated interaction energies in a quantum mechanical prediction of the paths that connect the reactants and the products and hence the distribution of the energy of the reaction among the possible quantum states of the products and the directions in which the products

will recoil (Schatz, G. & Kupperman, A.J. *J. chem. Phys.* **65**, 4668; 1976).

That these predictions have remained untested until now is in part because there is a barrier of 0.42 eV to be overcome before reaction can take place and in part because it is difficult to generate a sufficient concentration of hydrogen atoms of high enough energy. Moreover, H_2 is a difficult molecule to detect spectroscopically. To surmount those barriers, two US research groups have now used laser technology to produce fast H atoms in the presence of D_2 to study the energy of the resulting HD product from the reaction $H + D_2 \rightarrow HD + D$. Both groups generated the H atoms by photodissociating hydrogen iodide in the presence of deuterium, D_2 , in the gas phase at a low pressure using the output of a high-power pulsed ultraviolet laser at 266 nm. The production of HD was measured within 5–100 ns after the photolysis laser pulse to ensure that it had no time to undergo any further collisions.

The two groups employed different methods to detect the energy states of the HD product. One, led by R.N. Zare (Stanford University), used a technique in which the HD absorbs several photons from a

tunable pulsed ultraviolet laser and becomes ionized; the results were reported at the XIIth International Conference on the Physics of Electronic and Atomic Collisions, in Berlin last summer. The other group, led by J.J. Valentini (Los Alamos National Laboratory), used a form of Raman spectroscopy (Gerrity, D.P. & Valentini, J.J. *J. chem. Phys.* **79**, 5202; 1983). Both methods give information on the relative populations of the rotational and vibrational energy levels of the HD product. As can be seen from the figure, there is close agreement between the results of the two groups. HD is produced principally in its lowest vibrational level ($v=0$) with rotational distributions that peak at low quantum numbers. About 75 per cent of the energy available in the reaction is converted into kinetic energy of the reaction products as they fly apart.

The experiments represent a breakthrough in the study of chemical kinetics because they yield the first detailed information for a member of the $H + H_2$ family of reactions and demonstrate the feasibility of studying such fundamentally important reactions. This work will undoubtedly provide the stimulus for even more refined theoretical studies and advance our understanding of the basic principles of chemical kinetics. □

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Immunology

Rational design of vaccines

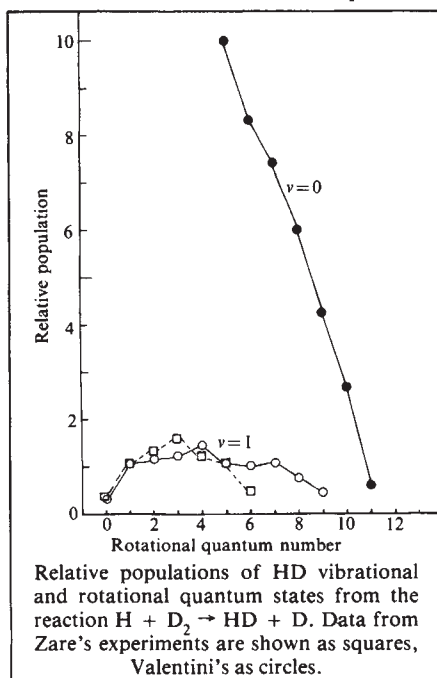
from N.A. Mitchison

THE late R.K. Gershon's major scientific legacy is to have demonstrated that the immune response, however simple it may look, is invariably the outcome of positive and negative regulatory influences mediated by helper and suppressor T cells. He showed that the key to understanding this balance is the experimental separation of the various helper and suppressor lymphocyte subsets, by means of alloantisera (such as anti-Lyt 2), monoclonal antibodies (such as T4 or T8), or lectins (for example, one from *Vicia villosa* reactive selectively with contrasuppressor cells), so that the regulatory properties of each cell type can be studied independently. Nowhere is this better illustrated than in the analysis of peripheral blood lymphocytes from patients with leprosy, reported by Mehra and his colleagues¹ in this issue of *Nature* (p.194).

Partitioning of these cells by means of T8 antibody reveals the presence, in lepromatous individuals, of a proliferative-responder putatively helper T-cell population which is prevented from acting by suppressor T cells. This form of suppression offers a rational explanation

for the continued progress of the disease in such cases. The determinant on *Mycobacterium leprae* with which the suppressor cells react is identified as a unique phenolic glycolipid. It can reasonably be assumed that the major infectious diseases afflicting the Third World, all of which are characterized by long-term interaction between host and parasite, involve a balance of this sort between regulatory lymphocytes. And if we knew how to design molecules able selectively to stimulate helper cells (or other positively acting regulatory cells such as contrasuppressors), we would be much further on the road to rational development of vaccines.

In a forthcoming paper, E. Sercarz and his group at the University of California, Los Angeles, bring their studies on lysozyme to a climax, with a definitive demonstration of a suppressor determinant at the N terminus of the protein². Using a bacterial protease they cleaved off the three N-terminal amino acids, leaving a molecule that was unable to induce suppressor T cells under defined experimental conditions. Using slightly less direct methods, J. Levy and co-workers have



similarly identified suppressor determinants in the ferredoxin molecule³.

This work should be seen in the context of a long series of studies which have identified particular molecules or parts of molecules as selectively interacting with distinct sets of lymphocytes. It traces back to the pioneering work of Leskowitz⁴ and Goodman⁵ on the extraordinary selectivity for T cells of arsenyl conjugates. Thus when guinea pigs are immunized with a two-headed synthetic antigen which has an arsenyl group at one end and a dinitrophenyl group at the other, the former attracts nearly all the T-cell response and the latter that of B cells⁵.

From the point of view of vaccine design, the interesting question that arises is why particular structures evoke suppression. There are two possible explanations: either there are structural features which cause a particular type of processing of a determinant within the immune system, or the receptor repertoires of T-cell subsets are different and therefore detect distinct determinants. The first possibility would be compatible with the mechanism preferred by Sercarz, whereby the location of an epitope close to an appropriate 'agritope' would cause selective recognition of the epitope by a particular T-cell subset. 'Agritope' is used here in the sense defined by R. Schwartz, as the part of an antigen which binds to the molecule of the major histocompatibility complex (MHC) responsible for presentation of that antigen (the 'agritope' binds to the 'desetope' on the MHC molecule, desetope being an acronym for determinant-selecting structure).

This brings us to one of the current immunological controversies: selective

antigen-presentation versus selective T-cell repertoires. In this sense Sercarz belongs to a school of thought dominant in the USA which espouses determinant selection as an explanation for immune response genes, in contrast to others who espouse repertoire selection. Evidence is accumulating that both mechanisms may operate⁶⁻⁸.

The most likely cause of differences between the receptor repertoires of helper and suppressor cells must surely be self-tolerance. After all, in the design of the immune system, there is an over-riding need for tolerance in the helper-cell compartment, but if anything the reverse is true for suppressor cells. At present we know next to nothing about tolerance of

self in suppressor cells, and it is the purpose of this article to draw attention to this gap in our knowledge. It should not be difficult to rectify.

If Sercarz is right, and structural features of a general nature can be identified in, or near to, suppressor epitopes, that would be good news for the Third World, for we could then hope to discover the rules of vaccine design. But if he is wrong, and there are repertoire differences mainly reflecting chance cross-reactions with self molecules, there may be no general rules, and that would be bad news. □

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Astronomy

Geminga — the source that is not there

from A.J. Dean

FIVE papers published between pages 158 and 166 of this issue of *Nature* seem to rule out the unpublished suggestion of P. Delache and his colleagues reported in these columns (*Nature* 305, 665; 1983) that Geminga, a rather mysterious cosmic γ -ray source, might also be exciting oscillations within the Sun by means of gravitational radiation. That suggestion, which would have had profound implications for several areas of astronomy, was based on a coincidence in periodicity between the 160-minute solar oscillation and a claimed periodicity (now discounted by the Caravane collaboration*) in Geminga's γ -ray emission. The five refutations of the suggested mechanism are based on analysis of gravitational wave propagation and on the solar response to such waves, and to that extent speak for themselves. But, apart from all this polemic, what accounts for the considerable current interest in Geminga itself?

Geminga is a veritable γ -ray machine. More than 99 per cent of its power output is observed in the γ -ray spectral range and it has the rare distinction that associated op-

tical, X-ray and γ -ray measurements are based on comparable numbers of photons. Discovered 10 years ago by the SAS-II satellite, Geminga has continued to resist positive identification with candidate counterparts at other wavelengths. Even now, after a series of deep surveys in a number of wavebands, it is impossible to discriminate categorically between a neutron star contender at 100 pc and a γ -ray quasar at $\sim 5,000$ Mpc. Interest will be intensified by a recent discovery by the French astrophysicist P. Durouchoux while guest observer of A. Jacobson on the JPL HEAO C-1 telescope. His preliminary analysis of data obtained in 1979 and 1980 suggests that Geminga is a powerful and variable (over a 6-month period) emitter of low-energy (1 MeV) γ rays.

It is the poor angular resolution of contemporary γ -ray telescopes that creates the crisis of identity for Geminga. Even the repeated COS-B observations have been unable to pinpoint Geminga to a region of sky better than a 0.4° error circle, rendering direct positional identification impracticable. Current efforts concentrate on the technique by which the Crab and Vela pulsars were identified — attempts to correlate γ -ray time signatures with flux variations from accurately located counterparts at other wavelengths.

A number of searches for Geminga's counterpart have in recent years yielded some exciting possibilities. After studying the relevant region of the sky with the imaging proportional counter on the Einstein satellite, G. Bignami, P. Caraveo and R. Lamb favoured identification with the brightest X-ray object in the field of view

*A telex dated 16 February 1984 from L. Scarsci says "With reference to the claimed detection of a 160-minute period in the γ -ray emission from the source 2CG195+04 (Geminga), the Caravane collaboration for the COS-B satellite wishes it to be known that, using their well tried analysis procedures, they do not find the result to be statistically significant".



100 years ago

THE latest official report on the conditions of the districts overwhelmed by the Krakatoa eruption states that the surviving inhabitants of the various villages have reassembled under their headmen, and are erecting their huts. The volcanic ashes did little harm to the soil, the growing crops all presenting a luxuriant appearance. The trees, however, have suffered greatly,

as had some of the coffee plantations. Two bays, Lampong and Semengka, which were blocked up by the fields of pumice, were free by the middle of December.

ON a summer night of 1882 a woman in Högsby parish, in Sweden, saw a shining object fall from the sky, disappearing behind a stable. Search was made for the meteorite, according to the statements of the woman, but without success. Last autumn it was, however, accidentally discovered near the spot indicated, and has now been forwarded to proper quarters in the town of Oskarshamn. The surface of the meteorite appears as if it had been welded from various substances; it is about the size of a billycock hat, very thick, and weighs a little over 14 lbs.

From *Nature* 29, 437, 6 March 1884.

(1E 0630 + 178), subsequently located to 3 arc s using the high-resolution imager on the same telescope system. Most of the emitted X-ray photons have energies below 1 keV with little evidence for interstellar absorption, indicating that the X-ray object cannot be more than 200 pc distant. A subsequent 10-hour observation with EXOSAT has confirmed the soft nature of the X-ray spectrum and a search for time variability is under way. A series of optical studies for this region of the sky has revealed a 21.3-magnitude point-like object, which is located at the edge of the error box of the Einstein satellite and which has an apparent lack of emission and absorption features, reminiscent of the Crab and Vela pulsars. Although the corresponding position on the Palomar sky survey is blank, a search of the 1955 Palomar plates by J. Bloeman of the Huygen's Observatory at Leiden has revealed an object, some 10 arc s away, which is not present in recent more sensitive (CCD) plates. The magnitude ($M_R = 20-20.5$) of the 1955 object is similar to that of the Einstein satellite's counterpart; if they are the same object, a proper motion of $0.37 \text{ arc s yr}^{-1}$ and a distance of 100 pc

are implied. By contrast, a series of astrometric observations between November 1982 and October 1983 by J. Lecacheux and co-workers has set an upper limit of $0.2 \text{ arc s yr}^{-1}$ on the proper motion of this object.

What is this source? The combination of its point-like nature and high ($\sim 1,000$) ratio of X-ray to optical luminosity rules out most known classes of X-ray objects, with the exception of low-mass binary systems and pulsars. The former seems unlikely because the measured low visual magnitude would place it at a distance of $\sim 250 \text{ kpc}$ which is incompatible with the lack of X-ray absorption. In any case, a pulsar is a more attractive orthodox candidate because the two galactic COS-B sources so far identified are pulsars and both show excess γ -ray luminosities.

Paradoxically, radio searches of the error box of the COS-B satellite have pinpointed a number of possible counterparts, none of which pulsates or coincides with the source identified by the Einstein satellite. One promising radio candidate, discovered using the 100-m Effelsberg telescope, has subsequently been identified

with the quasar 0630+180 by the SAO/UAO Multiple Mirror Telescope. Should this distant quasar prove to be Geminga, at $L_\gamma \geq 10^{49} \text{ erg s}^{-1}$, it would be the most luminous source of high-energy photons known, assuming isotropic emission. Alternatively, if it is a highly collimated γ -ray jet beamed towards the Earth, its luminosity would not be so great.

Although there is still no solution to the riddle of Geminga ("the source that is not there" in Milanese dialect), the detailed multi-waveband searches have demonstrated that it is dominated by γ -ray emission. Furthermore, whether a neutron star or a quasar, it is highly likely that Geminga offers the uncommon possibility of looking straight down a cosmic γ -ray beam. Finally, it is clear that, in the absence of temporal correlations across the wavebands, the question of what is the counterpart to Geminga will only be answered by use of a future γ -ray telescope with much improved angular resolution. $\square$

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Astronomy

IRAS circulars 8 and 9

The source name consists of four parts: (1) the letters 'IRAS' to indicate the origin; (2) right ascension (RA) in hours and minutes and seconds; (3) declination (Dec) in decimal degrees, multiplied by 10 and then truncated (i.e. $+32 \text{ deg } 42.3 \text{ arc min}$ becomes $+327$); (4) an appendix starting with 'P' and followed by the number of the circular; this appendix stresses that the data are preliminary. Position is given at equinox 1950.0. The measurements have been made between epochs 1983.1 and 1983.4 (for circular 8, below left) and between epochs 1983.1 and 1983.7 (for circular 9, below right).

The sources in circular 9 (below right) were selected because their flux densities have ratios compatible with those of the OH IR star discussed in a forthcoming paper in the *Astrophysical Journal Letters* (15 March 1984) by Olmon *et al.* The list may contain planetary nebulae. The sometimes very large upper limits to the $100\text{-}\mu\text{m}$ fluxes are due to high background at low galactic latitudes. Uncertain flux values are marked*. The sources were selected by H.I. Habing and F.M. Olmon, Sterrewacht, Leiden, Netherlands.

Source IRAS	RA h min s	Dec deg arc min	Flux density (Jy)	Source IRAS	RA h min s	Dec deg arc min	Flux density (Jy)
			12 μm 25 μm 60 μm 100 μm				12 μm 25 μm 60 μm 100 μm
0412+287P08	04 12 25	+28 40.3	<0.4	0017+657P09	00 17 07	+65 42.9	26 37 8 6
0437+257P08	04 36 52	+25 39.2	4.8	0021+623P09	00 21 05	+62 21.5	45 54 14 7
0503+316P08	05 03 06	+3 36.0	<0.3	0113+645P09	01 13 19	+64 34.9	4.2 49 141 125
0513+455P08	05 13 07	+45 30.8	26	0244+693P09	02 44 08	+69 23.0	12 23 18 21
0621+495P08	06 21 04	+49 32.2	<0.3	1912+172P09	19 12 46	+17 17.3	12 20 10 <11
1725+050P08	17 25 40	+05 04.7	17	1913+215P09	19 13 26	+21 31.2	4.8 16.4 9.9 <5
1730+083P08	17 30 49	+08 22.7	12	1917+199P09	19 17 18	+19 56.1	4.6 7.5 2.5 <10
1744+307P08	17 44 35	+30 43.3	<0.6	1920+156P09	19 20 02	+15 36.0	6.4 12 6.6 <36
1756+062P08	17 56 59	+06 17.4	<0.4	1920+210P09	19 20 05	+21 01.5	10.9 27 12 <8
1806+241P08	18 06 16	+24 10.1	3.8	1922+302P09	19 22 29	+30 13.5	1.0 2.7 1.4 <3
1806+091P08	18 06 55	+09 11.7	66	1923+164P09	19 23 26	+16 27.1	0.9 8.0 17.3 <18
1807+347P08	18 07 37	+34 45.6	28	1923+167P09	19 23 39	+16 47.5	0.9 8.7 7.5 <16
1809+015P08	18 09 05	+01 30.9	<0.3	1928+293P09	19 28 51	+29 23.6	37 61 18 9.4*
1809+270P08	18 09 31	+27 04.5	43	1930+141P09	19 30 37	+14 07.1	3.6 63 35 13
1809+149P08	18 09 35	+14 58.1	0.91	1937+239P09	19 37 28	+23 59.3	21 105 81 <9
1812+051P08	18 12 21	+05 11.9	10	1938+152P09	19 38 37	+15 13.1	35 35 5.9 <3
1813+067P08	18 13 37	+06 43.7	<0.2	1938+154P09	19 38 46	+15 27.2	6.2 7.0 1.5 <3
1814+220P08	18 14 34	+22 05.6	<0.3	1944+228P09	19 44 01	+22 52.0	15 30 14 <9
1823+089P08	18 23 10	+08 55.0	4.6	1945+293P09	19 45 24	+29 20.7	16 95 64 18
1823+218P08	18 23 43	+21 50.4	6.6	1945+172P09	19 45 55	+17 16.5	5.4 7.1 1.8 <2
1824+012P08	18 24 37	+01 12.6	0.4	1947+240P09	19 47 48	+24 01.2	10.0 58 31 <6
1825+078P08	18 25 26	+07 50.4	5.6	1952+279P09	19 52 03	+27 59.7	44 125 240 282
1826+227P08	18 26 18	+22 42.1	<0.3	1953+280P09	19 53 28	+28 02.8	8.1 14 4.0 <10
1826+012P08	18 26 59	+01 16.6	3.2	1954+305P09	19 54 49	+30 35.9	70 115 47 15
1833+055P08	18 33 19	+05 33.3	280	1955+335P09	19 55 54	+33 33.2	43 52 15 <14
1850-796P08	18 50 18	-79 37.8	<0.2	2005+185P09	20 05 40	+18 34.2	16 19 6 3
1905-750P08	19 05 06	-75 02.3	7.4	2010+308P09	20 10 23	+30 53.9	3.1 6.2 10.2 20.1*
1927-746P08	19 27 31	-74 39.4	<0.3	2013+286P09	20 13 44	+28 38.6	4.1 9.3 2.6 <4
				2016+275P09	20 16 01	+27 34.6	0.8 2.0 2.0 <3
				2018+225P09	20 18 11	+22 34.2	25 34 8.0 <2
				2326+689P09	23 26 49	+68 54.3	26 38 49 23
				2332+657P09	23 32 07	+65 45.3	13 90 76 25

Mathematics for the thousands

Mathematics will increasingly need to be taught in subjects other than physics and engineering. Peter Saunders considers how mathematicians best can help.

LIKE other university teachers, mathematicians generally prefer teaching students who are specializing in their subject. Most of them also get considerable satisfaction from lecturing to physicists and engineers, who also show a high level of ability and motivation. Far fewer enjoy tackling one of the elementary mathematics courses that are put on for large groups of first-year students, most of whom will do no more on the subject at university. In the United States this is generally a freshman course in calculus and geometry; in Britain it is more likely to be an ancillary course for (say) biologists. Both are often seen as Cinderella courses, to be avoided where possible (perhaps even left for graduate assistants to teach) or else accepted as chores to be endured.

It is not hard to see why this is so. Many of the students will have given up mathematics at school as soon as they could; part of the reason that they chose their particular field of study may even have been that it allowed them to avoid mathematics. Yet no sooner do they arrive at university than they find themselves sent into a large room to be lectured at on the very subject they thought they had escaped. Many students do in fact learn quite a bit in these courses — but it is hardly an auspicious start.

A start must, however, be made, for the importance of mathematics extends far beyond its traditional preserve of the physical sciences and engineering. Mathematics is essentially the study of patterns,

... no sooner do they arrive at university than they find themselves sent into a large room to be lectured at on the very subject they thought they had escaped.

and so it has a place in any subject in which there are patterns to be explored — which means almost any subject at all, and certainly any subject which claims to be a science.

This has always been true in principle, but two factors are combining to make it a matter of immediate concern. First, as the biological and social sciences advance, more and more sub-disciplines are reaching the stage where the patterns that are being investigated are of such subtlety and complexity that they cannot be fully understood without mathematics. Correspondingly, mathematics has been making inroads into new fields. But the sudden upsurge in the availability and use of computers has brought about a dramatic speeding up of the process.

Until recently there were many problems

which could be expressed in mathematical terms but on which little or no progress could be made on account of the sheer complexity of the calculations. This was true in most subjects, but at least in the physical sciences there were enough tractable problems to keep applied mathematicians fully occupied. Most workers in other fields, however, could justify ignoring mathematics — not because it was irrelevant, but because using it was too difficult. The computer has now made mathematics just easy enough to use that these people have lost their excuse. So it is even more important than it was in the past for mathematicians to consider carefully how best we can help.

Aims. First we must understand what we are really trying to do. Above all, we must not set our sights too high. If we want to train mathematical biologists or mathematical economists, we will have to set up proper combined courses designed for the purpose. Naturally it would be best if all students could acquire some facility in handling mathematics, but the first aim must be to make sure that they have at least a passive knowledge of the subject. Not every biologist has to be able to set up a simple differential equation like the one representing the logistic growth of a population, but they should all understand what is meant by

$$dx/dt = rx(1 - x/K),$$

at least to the point that they can see the connection with the concepts of r - and K -selection. They may never quite be able to remember how to solve the equation, but they should know that it can be done, and preferably more or less how, and they should also know that there is an arbitrary constant to be chosen, and what its significance is.

Even those students who will never need to use much mathematics themselves can hardly avoid having to read papers or books that do. In preparing them for this we will also give them at least a bit of the advantage that mathematicians have in that if you study the patterns themselves you only have to do the work once. After that, if the same idea turns up you are ready for it. I can still recall how as an undergraduate I kept hearing about a mysterious concept called marginal utility which seemed to baffle my friends in the social sciences. Had they learned even a tiny amount of calculus, I dare say they wouldn't have found it at all hard to grasp.

It is not always easy to convince staff in other departments of this point. Some of them still think of time spent learning mathematics only as time lost from what really matters. I even know of one

department which gave as the reason for withdrawing students from an ancillary mathematics course the fact that it was no longer necessary as they would be studying epidemiology. I would have thought that this made it more necessary, not less, but perhaps the students don't mind not knowing what the funny symbols in the equations mean.

Subject matter. The choice of material is probably the easiest task. Of course, a lot depends on how much the students have already learned and how long ago this was,

Some staff still think of time spent learning mathematics only as time lost from what really matters.

and on how much time is available. But I think there is general agreement on the topics that ought to appear in an introductory course: a bit of coordinate geometry, calculus up to elementary differential equations, matrices and determinants, and some statistics. There should also be some numerical analysis; this was often omitted in the past but nowadays students must have at least some idea of how computers do their work, and how and why there can be problems about accuracy or even output which is sheer nonsense.

If the whole class is from the same department or subject area, the details of the material can be adjusted to suit, but there is less room here for manoeuvre than is generally thought. This is not just a matter of mathematicians being reluctant to allow anyone to tamper with the elegant structure of their discipline. Almost everyone finds it much easier to learn ideas which flow one from the other than to cope with a mere collection of unrelated statements apparently pulled out of thin air. So we must not be too readily seduced by the idea that it is desirable or even possible to teach only those particular bits of mathematics which are immediately relevant, or seen as such by other departments. I once sent around a questionnaire about an ancillary course I was teaching. One respondent urged me not to bother with differentiating sine and cosine, but to make sure that the students learned about simple harmonic motion. Not every case is so clear cut, but I think this illustrates the point.

Teaching: to prove or not to prove? Any problems there may be in selecting the material pale by comparison with actually doing the teaching. This can be very different indeed from any other lecturing, the most important difference being that most of the class are not interested in mathematics as such, although some of them will, probably much to their surprise, discover that they can enjoy it. So the lectures have to be well paced, and long calculations and detailed arguments avoided unless absolutely necessary. There must also be plenty of worked examples.

What the students want is to be told what the result "really" means, and how it is used (many lectures to specialist mathematicians could do with a bit more of this too; it is largely a question of degree).

This does not mean that all the proofs should be left out. Admittedly, no one will mind if the results are not established with full rigour, but proofs serve other purposes as well. They take some of the mystery out of mathematics, and they can help the students to understand theorems by showing them why they are true. They are even useful simply as a means of slowing the lectures down; without them it is all too easy to rush through topics far too quickly. A proof of one result is often a straightforward application of an earlier one, so while the class may think they are being shown something new the lecturer's chief aim may be to reinforce something they have already seen.

Of course we then have to remember why we are going through some of the proofs, and must not let a misplaced sense of duty prevent us from omitting any that are too difficult or too long. In specialist courses the proofs are often the whole point of the lecture; here they are merely corroborative detail intended to give artistic verisimilitude to an otherwise bald and unconvincing narrative.

Examples. While everyone agrees that examples are especially important in courses for non-mathematicians, there can be a considerable difference of opinion

The students will generally demand examples which are "relevant". . .

when it comes to choosing them. The students — usually supported by the staff in their own departments — will generally demand examples which are "relevant", by which they mean drawn from their own fields. The lecturer, on the other hand, will be inclined to rely on the old war-horses taken mostly from physics.

There is a real difficulty here. It is certainly hard to convince a class that they ought to learn mathematics if none of the illustrations have anything to do with the subjects they are really interested in. On the other hand, most of the plausible, simple examples do come from physics. My own solution has been to draw on physics when introducing the basic ideas — most people have a sufficient understanding of what is meant by velocity and acceleration for them to be used to put across the concept of a derivative and a second derivative — and then to use "relevant" examples afterwards for reinforcement and motivation.

Motivation. It is hard for many students to see the point of any course outside their chosen subject, but the problem is even more acute with mathematics in biology and the social sciences. After all, most people working in these fields seem to be managing perfectly well without it, and it is not easy to convince students that even if this is possible today, it is unlikely to

remain so for the next 40 years. Some of the staff, too, may not see the need for mathematics, nor make any pretence about their view, although in my experience this attitude is far less common than it used to be. What is more likely is that a remark intended to reassure someone who is in real difficulties with mathematics may be passed on to the rest of the class and taken to mean that the subject isn't really important after all. In the same way, it would be unwise to adopt the suggestion that courses in mathematics for biologists should be non-examinable. Obviously no

There is a world of difference between being a competent user of some bits of mathematics and being able to design and teach an introductory course on the subject.

one would want failure in such courses to count too heavily, but once the students learn that there is no penalty for neglecting them, many will be all too ready to take the hint.

On the positive side, the best way the home departments can help is by making sure that at least some mathematics is used in the first year. However realistic an example may be, the mere fact that it appears in a mathematics course makes it suspect; exactly the same example, worked into a lecture in the field from which it comes, is far more convincing. So for an ancillary course to be successful, there must be active cooperation between the mathematicians and the departments whose students they are teaching. Most of the advantages of this are obvious, but I have also found that it is helpful for the students just to know that there is real collaboration, and that their department is not merely sending them off into the wilderness of mathematics as some form of initiation rite.

From this point of view, it might seem attractive for the home departments themselves to teach the mathematics courses. In fact, very few are equipped to do this properly. There is a world of difference between being a competent user of some bits of mathematics and being able to design and teach an introductory course on the subject. If a department is really convinced that the mathematicians are unable or unwilling to put on something suitable, then they may have to consider doing it themselves. But if their motive is mere empire building it is the students who will be the losers.

Mathematics for the thousands (if not quite the million). Courses in mathematics for non-mathematicians have traditionally been thought of in connection with the main field of study: statistics for biologists and social scientists, differential equations for engineers, nothing at all for students of English literature, and so on. But graduates have always followed careers far removed from what they have been

studying, and this is likely to become even more common. Changing careers during one's working life may even become the rule rather than the exception.

Because mathematics permeates so many fields, it is one part of students' training which is likely to remain useful throughout their working lives. Certainly this is true for the ever-growing proportion of students who will go into computing in one form or another, which both increases the importance of introductory courses and makes it undesirable that they should be tied too closely to specific needs.

There is, however, an even more important corollary. What is true for students in those disciplines for which some sort of mathematics teaching has traditionally been provided is true for others as well. If anything it is more so, for in the past science students have usually expected to carry on in science. It is the historians and the classicists who have always been more likely to treat their education as a general preparation for unrelated careers.

Just as science graduates have to be able to write understandable English, students in the humanities are also going to have to cope with an increasingly mathematical world. Universities have a duty to both groups. Many in the United States have already done something about this, an outstanding example being the "core curriculum" at Harvard. British universities have always tended to assume that whatever general education students require they will have received in the schools, but they may have to think again.

Some people are pessimistic about the future demand for mathematics in the universities. I do not share their view. At a time when the world is becoming more and more mathematical, it would be totally wrong for the universities to react by teaching less mathematics. Nor is the computer a substitute; on the contrary, it is a major factor in increasing the need for mathematics.

Perhaps there will be fewer students specializing in mathematics than in the past (though even here I expect that the changing attitude in the schools will be reflected in a much greater number of qualified applicants). But there is bound to be more need for combined honours courses, for ancillary courses and for introductory courses designed to ensure that all graduates are numerate as well as literate. So there will always be enough work for mathematicians to do. We may, however, find it necessary to adapt to changing needs, and one of the changes is likely to be that ancillary and introductory courses are going to take a larger proportion of our teaching effort. We may find such courses a challenge, but we had better get used to them. □

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Textbooks: a genetic selection

J.G.M. Shire

Understanding Genetics, 3rd Edn. By Norman V. Rothwell.

Oxford University Press: 1983. Pp. 647. \$28.95, £24.50.

Concepts of Genetics. By William S. Klug and Michael R. Cummings.

Charles E. Merrill: 1983. Pp. 614. Hbk \$29.95, £25.15; pbk £10.95.

Genetics. By George J. Brewer and Charles F. Sing.

Addison-Wesley: 1983. Pp. 729. \$28.95, £15.95.

Genetics. By John R.S. Fincham.

John Wright: 1983. Pp. 643. Pbk £12.50, \$32.50.

Problems on Quantitative Genetics. By D.S. Falconer.

Longman: 1983. Pp. 104. Pbk £3.95, \$7.95.

Theory and Problems of Genetics, 2nd Edn.

By William D. Stansfield.

McGraw-Hill: 1983. Pp. 392. Pbk \$7.95, £5.75.

DNA for Beginners.

By Israel Rosenfield, Edward Ziff and Borin van Loon.

Writers & Readers: 1983. Pp. 224. Pbk \$6.95, £2.95.

The Cartoon Guide to Genetics. By Larry Gonick and Mark Wheelis.

Barnes & Noble/Harper & Row: 1983. Pp. 215. Pbk \$5.25, £3.95.

IN THE late 1970s most publishers caught on to the vigorous growth of genetics and were encouraged in their plans by the relatively few textbooks then available. The consequence is that there are now a large number of texts, all competing for a share of the market. As the selection pressures intensify one looks increasingly for the books that are likely to thrive and for those likely to become extinct. Long-term success will require both adaptation to the current situation and flexibility to meet future changes in both the subject and the market.

The books under review fall into two phenotypic classes, four of them being between 32 and 36mm thick whilst the others are relatively slim (6–19mm). The first four volumes also resemble each other in weight (about 1.5kg) and in the excellence of their paper, typography and illustrations. Three of them show further resemblances. Rothwell, Klug and Cummings, and Brewer and Sing all originate in the United States and have effective, coloured artwork covers. All three have red highlighting of the text and diagrams, extensive glossaries and end each chapter with problems and selected further reading. Indeed their outward phenotypes are so similar, and so like those of existing texts, that it would seem that publishers choose the same idealized model to mimic. (The mimicry is unusual, being the inverse of aposematic, since the aim of each species is to maximize its predation by students.) Closer examination reveals real differences between the three, however, and thus in their chances of attracting university-level students taking a course or courses in genetics, but not necessarily specializing in the subject.

Rothwell's *Understanding Genetics*, now in its third edition, is a straightforward textbook devoted to transmission genetics and the study of the genetic material. It begins with Mendel's peas (though they

produce sperm) and proceeds down a familiar route through diploid analysis and cytogenetics before reaching DNA and microbial systems. It ends with population genetics and evolution. Interestingly, quantitative genetics is integrated with formal diploid analysis. Throughout there is an emphasis on the spatial organization of the genetic material, from introns and exons to the distribution of DNA amongst chromosomes and between organelles. In all, this is an up-to-date text for genetics courses organized on wholly conventional lines.

Concepts of Genetics by Klug and Cummings also adopts a predominantly historical order, though meiosis precedes Mendelism and DNA synthesis comes before microbial genetics. The book ends, predictably, with population genetics and the implications of recombinant DNA. A characteristic of this text is a lack of emphasis on numerical and quantitative data; analysis of continuous variation is covered in about a page-worth of text scattered through the book, despite an interesting 18-page chapter on behaviour genetics. The chapters on developmental genetics and somatic cell genetics are very readable and will arouse a student's interest. They reflect the tenor of the whole book, in which animal examples predominate. Similarly, passages such as "tall sperm fertilizing dwarf ova" (in an account of Mendel's peas on p.35) will not endear the book to students of plant biology. Despite the good points I cannot recommend it as a text even for animal biologists, for zoologists need to be numerate just as much as other biologists.

Brewer and Sing's *Genetics* is the most interesting of the subset of three books. The first two chapters combine short case studies with an exposition of the basic facts of genetics and can be commended to anyone who wants a simple but informative introduction to the subject. These chapters

also include an explicit justification for adopting a "DNA-first" (see *Nature* 289, 702; 1981) scheme for the main body of the book. Linkage is introduced as a consequence of chromosomal structure, preceding the discussion of diploid genetics which is not reached until the eighth of the sixteen chapters. As a refreshing change, mice provide the examples, and lead on to a discussion of complementation in diploids and the *cis-trans* test in microorganisms; this is a fruitful combination since many students have difficulty relating these two aspects of the same basic concept. Brewer and Sing draw examples from human genetics when possible and have a good section on the immune system. Surprisingly, though, they hardly mention somatic cell genetics or the analysis of behavioural traits.

The book does have some defects. For example, the presentation of what happens to chromatids during crossing-over will permanently confuse many undergraduates. On balance I feel that Brewer and Sing's brave attempt does not quite succeed, though some rapid directed mutation could lead to a truly different and competitive textbook.

The photomicrograph of mitotic prophase on the cover of Fincham's *Genetics* differentiates it from the other large books. Between the covers, major differences in approach, level and content are revealed. The book gives an account of genetics as it is today, not as it has developed historically. DNA structure precedes meiosis, and the account of transmission genetics starts with the analysis of ordered tetrads in fungi before moving on to the analysis of random meiotic products. Diploid ratios emerge as a corollary. Mapping and recombination are also firmly based on tetrad and half-tetrad analysis. There are strong sections on microbial systems and recombinant DNA technology, while parasexual analysis, cytogenetics, quantitative genetics and differentiation are all covered within the overall context of genetic organization and its evolution. This theme culminates in the final two chapters, which discuss evolution and human genetics.

Fincham's book should stimulate all geneticists and all students who intend to become geneticists or molecular biologists, but will be too strong meat for those seeking elucidation of their introductory lectures in genetics. It is not a direct competitor of the other three textbooks, or of the current front-runners in the race for students' money — Ayala and Kiger's *Modern Genetics* and Suzuki, Griffiths and Lewontin's *An Introduction to Genetic Analysis* (for reviews see *Nature* 289, 702; 1981 and 295, 476; 1982) — but stands out as a book which geneticists of all kinds should acquire.

The slimmer books may also be divided into distinct categories. Two are based on the premise that numerical problem-solving is basic to an understanding of genetics. Falconer's book is a useful

companion to the second edition of his *Introduction to Quantitative Genetics* (for review see *Nature* 295, 475; 1982), and individual problems are cross-referenced to the main text. He has devised a novel and effective numbering system which prevents one seeing the solution to the following problem, even accidentally or subliminally. He gives more space to the answers than to the questions, while in Stansfield's *Theory and Problems of Genetics* the answers are a third of the length of the problems. In this second book each set of problems is introduced by some notes punctuated with definitions and worked examples. While Stansfield's book will be a helpful source for teachers requiring new problems — those on DNA and molecular genetics being particularly welcome — its format will not make it attractive to students nor will its small type and poor paper.

The last two books are competing for the same host: people who buy cartoon books in the hope of absorbing knowledge painlessly. In *DNA for Beginners*, Borin van Loon, the illustrator, evidently gained the

upper hand, even to the extent of providing a self-portrait. There are some striking montages and most of the famous scientists are recognizable but the overall presentation of the cell is overly mechanical; all the enzymes are variants of bulldozers or locomotives. Even so, the book will raise a chuckle amongst molecular biologists of all kinds. The artwork of Gonick and Wheelis's little paperback is less polished but the storyline flows much better. The models are simpler and more molecular: there is a charming RNA polymerase beast which has to work hard for its living. Despite a basic confusion over crossing-over, the book covers transmission genetics, genetic engineering and its social consequences effectively. *The Cartoon Guide to Genetics* is fun and can be recommended to people asking for a simple introduction to genetics and molecular biology. It can be likened to a useful weed — highly successful in the short term but ultimately ephemeral. □

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Molecular intrigue

W.J. Brammar

Molecular Biology: A Comprehensive Introduction to Prokaryotes and Eukaryotes.

By David Freifelder.

Jones & Bartlett, 20 Park Plaza, Boston, MA: 1983. Pp.979. \$35.

Companion workbook \$8.95.

Genes. By Benjamin Lewin.

Wiley: 1983. Pp.715. \$43.85, £27.35.

THE exciting developments in our understanding of molecular biology, together with the potential for biotechnological exploitation, have made the subject very popular with students and created a continual need for predigested information in the form of reviews and textbooks. Well-written texts can make an invaluable contribution to the development of a subject through their ability to influence large numbers of future practitioners. The two new books reviewed here have the quality to make a significant impact in molecular biology, though it is likely to be temporary given the pace of discovery in the field.

David Freifelder's *Molecular Biology* is based on a course taught by the author at Brandeis University and attempts a broad coverage of molecular biology at an intermediate level. Freifelder's philosophy is that "molecular biology must emphasize both molecules and biology and that to be molecular it must also be chemical and physical". Although this accurately reflects the approach taken, biologists without an advanced training in chemistry and physics should not be alarmed; the

chemical and physical principles are unobtrusive and are introduced only to clarify the discussion of molecular phenomena.

The first of the 22 chapters explains the logical and methodological approach to molecular biology and introduces those prokaryotic and eukaryotic systems that are the main subjects for molecular analysis. Freifelder's belief in the value of the genetic approach alongside *in vitro* biochemical and physical techniques, and in the wisdom of choosing relatively simple systems with which to establish principles, is apparent at this early stage.

The second chapter provides a parallel introduction to basic biochemical concepts, including metabolic pathways and energy metabolism, before Freifelder launches into the accounts of the structures and properties of macromolecules and the methods used to study them, and then consideration of nucleic acids, proteins and macromolecular aggregates. DNA replication, repair and mutagenesis each have their own chapters, while translation merits 110 pages in two chapters on "The Information Problem" and "The Machinery of Protein Synthesis". Recombination, bacteriophages, plasmids, animal and plant viruses and the control of gene expression are all treated in similar detail.

Freifelder is remarkably successful in achieving his aims. He manages to remain authoritative throughout the book despite its wide coverage, and he has the gifted teacher's ability to make the material seem simple and the way ahead challenging but full of promise. The clear text is augmented by many well-designed, two-tone illustrations and some excellent photographs and electron micrographs. The legends are occasionally at variance with the diagrams,

but the errors are few and usually trivial. *Molecular Biology* will be an excellent source-book for students. I anticipate that the coming years will see a long line of new editions.

In *Genes*, the latest of Benjamin Lewin's series of successful molecular genetics texts, the author has set himself the task of distilling the mass of recent information "to discern general principles and describe the state of the art". This is a forbiddingly difficult goal in a field that is progressing so rapidly. But a *modus operandi* that allows him to assemble text alongside or ahead of the current literature and remarkable alacrity by the publisher have resulted in its complete achievement.

Lewin contends that *Genes* is "simply about genes, recognizing what amounts to a new field whose extraordinary progress has all but overwhelmed the traditional discipline of genetics". Many would interpret "overwhelmed" as hyperbole and readers of John Fincham's excellent new textbook, *Genetics* (for review see p.117), will appreciate the validity and value of interweaving the new, molecular information with the existing tenets of the subject to produce a lively and cohesive discipline still recognizable as genetics. Indeed, one of the strengths of Lewin's book is the frequent illumination of the phenomena of classical genetics by molecular information.

Lewin assumes no prior knowledge of molecular biology, providing clear introductory chapters on the gene from the genetic and biochemical viewpoints. With an abrupt change of pace we are introduced within ten pages to restriction mapping, DNA sequencing, interrupted and overlapping genes. This sets the tone for the rest of the book, which deals in remarkable detail with all aspects of gene structure and function. Prokaryotic and eukaryotic systems are given equal weight, with all the major experimental systems being considered. The treatment is fully contemporary with the 1983 literature and its depth is well suited to the advanced undergraduate student. Where conclusions require qualification, Lewin provides a full and balanced discussion of the alternative possibilities. Each chapter concludes with a short bibliography largely confined to review articles.

Lewin's style is vigorous and clear, providing information succinctly and without fuss. All the important points are illustrated by helpful diagrams, drawn to scale in several tones, while a 14-page glossary is provided to minimize the usual problem of terminology. The story of molecular genetics is now an intriguing one to tell, and in *Genes* Lewin relates it with great enthusiasm and skill. I would advise all students of molecular genetics to procure a personal copy and to assimilate its contents. □

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Genetics and ethics

Ben Carritt

Human Genetics. By Daniel L. Hartl.
Harper & Row: 1983. Pp. 605.
\$33.95, £10.50.

Human Genetics. By John B. Jenkins.
Benjamin-Cummings: 1983.
Pp. 461. \$22.95, £22.05.

How many geneticists would select Man as the material to illustrate the principles of their subject? Many teachers, I would guess, whilst recognizing the enormous public and clinical interest in human genetics, would prefer to treat the subject as a subsidiary option, available only to those who had first mastered the fundamental concepts as exemplified by more lowly organisms. Neither of the authors of these two books would agree with that view. Thus Hartl: "Human genetics [is] an ideal theme for teaching virtually all aspects of genetics".

Both books, therefore, have the difficult task of guiding the student from almost (Hartl) or literally (Jenkins) no knowledge of genetics, biology or chemistry towards an understanding of the complexities of modern human genetics. Each takes a different route, and neither seems illogical as presented by its author. Hartl starts with the cell, leading through mitosis, chromosomes and meiosis to Mendel's laws, before tackling gene structure and function, and population, evolutionary and quantitative genetics. The introductory chapters are clearly written, especially that dealing with recessive inheritance, except for the

unhelpful details on cell fusion. Hartl's account of chromosome abnormalities is particularly well presented (although I could not make out what "Y chromosome monosomy" is), and here, as elsewhere, there are pertinent and interesting case examples. The chapter on immunity and blood groups is also good reading, but that dealing with the regulation of gene expression I found confusing and mostly irrelevant to human genetics. Likewise, the rather sketchy chapter on viruses.

Jenkins's book is pitched at a somewhat lower level, being aimed at the general student with "no knowledge of college level biology or chemistry". Human genetics, says Jenkins, although "fun", is "subject to misinterpretation and abuse. When people cite genetics in support of social ideas we must be quite sure that they know what they are talking about. . .". The avuncular voice and liberal sentiment are used to good effect throughout the book; and while Jenkins's direct and informal style may not find favour in the more austere European establishments, it is one that his younger readers no doubt take for granted. However, the tabloid prose to be found in some of the boxed sections designed to "highlight a variety of subjects" was often too much for me.

Both authors invite us frequently to form opinions on the wider implications of advances in human genetics and, indeed, provide discussion points on ethical points in their set problems at the close of each chapter. Hartl is generally content to pose the moral question without offering his own solution, beyond the reference to literary, Biblical or Chinese texts. I did not find these especially illuminating. Jenkins, on

the other hand, is more forthright, and rarely fails to guide us on these matters in a humanistic way.

Human genetics emerged from its "backwater" (Hartl) largely as a result of the twin technologies of somatic cell genetics and recombinant DNA. The difficulty of doing meaningful genetic studies in Man using traditional approaches, together with the pressing nature of our ignorance in many important areas, were catalytic factors in the development of these two techniques. And yet, the treatment of each is scant and occasionally incorrect in both books. Indeed, Hartl seems unconvinced about the ultimate usefulness or desirability of recombinant DNA research, surely a somewhat idiosyncratic stance.

Both books are well produced, with many helpful illustrations. In both, key words are highlighted in bold type, and in Hartl important sentences are also picked out in italics. Both also provide a summary at the end of each chapter and a glossary at the end of the book. I cannot see the point of this, as it is surely the purpose of the book to make such lists of definitions unnecessary. In any event, definitions such as that for DNA as "the chemical basis of heredity" as found in Jenkins's glossary are not particularly enlightening. □

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Fine fare

Richard Batchelor

Immunogenetics.

By Marek B. Zaleski, Stanislaw Dubiski, Edward G. Niles and Roger K. Cunningham.
Pitman: 1983. Pp. 514. £18.75, \$34.95.

AT A recent immunogenetics meeting attended by people from a number of different disciplines, strong criticisms were heard to the effect that the molecular geneticists were only talking to each other. Although there may have been a germ of truth in the complaint, a stroll to the bookshop would have been more productive than berating the geneticists. A few hours spent reading *Immunogenetics* would have provided the necessary background for dialogue.

Dr Zaleski and his colleagues take the reader easily through the basic principles of genetics, including a remarkably clear and up-to-date account of the molecular side of the subject, before turning to the main dishes on their menu. These include

immunoglobulins, blood groups, major histocompatibility systems, and cell surface alloantigens of nucleated cells. Structure and function are covered as well as genetics. The expositions of the different topics are clear and accurate, the illustrations and tables both thoughtful and useful. But what I particularly enjoyed were the well-informed, historical introductions; graduate students preparing the outlines of their theses should find these very useful.

The authors are well aware of the difficulties of writing about rapidly advancing subjects, and of course they have chosen a hornets nest in this respect. For example, within the last couple of months a full molecular map of the HLA region complement genes has been published, and this makes the relevant section of the book appear inadequate. Nevertheless such problems are inherent in the writing of textbooks. We should be grateful that in *Immunogenetics* Marek Zaleski and his co-authors have achieved so much. □

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Recombination: tale and technique

I. Barry Holland

Recombinant DNA: A Short Course.

By James D. Watson, John Tooze and David T. Kurtz.

W. H. Freeman: 1983. Pp.260.

Hbk \$27.95, £25.95; pbk \$17.95, £15.50.

Recombinant DNA Techniques: An Introduction.

By Raymond L. Rodriguez and Robert C. Tait.

Addison-Wesley: 1983. Pp.236.

Pbk \$21.95, £16.95.

In *Recombinant DNA*, James Watson and his co-authors have come up with a delightful book, not only to read but to look at since it is brilliantly illustrated in the *Scientific American* mould. The major developments in the story of molecular biology are succinctly put in their historical context, clearly and with no frills, leaving the reader free to pursue the underlying evidence from the reading lists provided.

Importantly, coverage is well up to date, the book including most of what the general biologist and first-year undergraduate would want to know about the principles of the recombinant DNA approach to research. How this approach is paying off is exemplified by accounts of antibody formation, transposable elements in pro- and eukaryotes, tumour viruses and the basis of cancer and other human diseases. The authors then move more specifically into the exploitation of recombinant DNA technology for application in biotechnology. This material is again presented incisively and with great polish, but almost exclusively in terms of cloning, manipulating and re-implanting mammalian genes.

In the final chapter on specific industrial applications, the scope is even more exclusive, being restricted to the production of viral and human proteins by *Escherichia coli*. This limits the value of the book to some extent, since clearly this is but the tip of the iceberg of the potential of the techniques and genetic systems so exquisitely portrayed in the earlier chapters. This chapter is also marred by the misconception that *E. coli* secretes proteins into the medium as a matter of course — while biotechnologists might wish that this were the case, the molecular biologist still finds it a challenging problem that only very rare proteins have the ingenuity to negotiate the double membrane of *E. coli*. But, in all, this is a captivating book which should (and will) be read by a far wider range of people than undergraduate students.

Despite the similarity of the titles, Rodriguez and Tait's *Recombinant DNA Techniques* is an entirely different book. In

it, the authors have set out to provide guidance in basic techniques for advanced undergraduates, post-graduates and others just beginning to get to grips with recombinant DNA technology.

Techniques in modern molecular biology are dangerously addictive, often more fascinating than some of the questions they are designed to illuminate. Moreover a laboratory may have just one method, refined to perfection, which stubbornly refuses to work in your own laboratory. Whole careers may be felt to depend upon the acquisition of the latest plasmid mini-prep procedure, sequencing short cut, cloning protocol or gene expression system — hence the enthusiasm to acquire manuals of techniques on the one hand and to write them on the other. Herein lies the rub! How does one present a topic, inherently boring when read, in a form and style which will engender sufficient comprehension to convince the inquisitive practitioner to sample the procedures?

Rodriguez and Tait's book consists of a series of lengthy discussions of some very basic cloning procedures — isolation and ligation of DNA, bacterial transformation and gel electrophoresis, for example — punctuated by exercises based upon the

cloning and characterization of *E. coli* genes in general and the histidine and arabinose operons in particular. The approach is descriptive and requires close attention on the part of the reader to follow the dense narrative style through to the practical diversions. Accessibility is not helped by the closely lined format of the text.

The exercises themselves are more clearly laid out in recipe style, but a drawback is that there is a tendency to assume that many of the experiments will be approached sequentially; there is little discussion of trouble-shooting or comparative evaluation of different techniques. There are useful lists of restriction and DNA modifying enzymes, plasmids with maps and sequences and bald protocols on DNA technologies (several of which are culled directly from earlier publications). Nevertheless this aspect (which takes one-third of the text) is the best feature of a book which otherwise suffers from a very specialist appeal and an apparent desire to be read rather than used. □

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Not too many cooks

John G. Edwards

Cell Biology: Structure, Biochemistry, and Function, 2nd Edn.

By Phillip Sheeler and Donald E.

Bianchi.

Wiley: 1983. Pp.668. £13.95, \$19.60.

Cell Biology, 3rd Edn.

By John W. Kimball.

Addison-Wesley: 1983. Pp.330.

\$25.95, £22.05.

Cells, 2nd Edn. By Michael W. Berns.

Saunders: 1983. Pp.256.

Pbk \$15.95, £7.95.

Laboratory Investigations in Cell Biology.

By Allyn A. Bregman.

Wiley: 1983. Pp.253.

Pbk £13.95, \$19.50.

FOR several years *Cell Biology* has been a fashionable label for textbooks which place an outline of biochemistry in juxtaposition with or (if you were lucky) in the context of cell structure. Very variable is the extent to which authors have really shifted cells to centre stage by telling us about the sorts of things they do. My suspicion, reinforced by reading the three new editions reviewed here, is that the range and rate of change of such material is too great, however heroic the attempt, for packaging by one or even two authors.

So the main development to consider since the previous editions of the three texts is not so much the advance of test-tube

genetics, which each has set out to accommodate, but the arrival on the scene of the six-author *Molecular Biology of the Cell* by Bruce Alberts *et al.* (Garland, 1983; for review see *Nature* 302, 637; 1983). This book has shown for the first time the full potential of cell-centred biological education.

The view downwards, from cells through organelles (impressively handled) to molecules, is dominant in Sheeler and Bianchi. The book has very good diagrams, a useful chapter on research tools (especially radioisotopes and cell-fractionation) and a newly added chapter on molecular genetics. The problem of range of material naturally shows most in the handling of grey areas. The authors occasionally slip into representing hypothesis as fact; no hint here that the microtubular lattice is controversial, that the tilting of myosin on actin is still model rather than measurement, or that contact inhibition is probably not what it seems.

Kimball's niche is distinctly different, the book consisting chiefly of the sections on biochemistry and genetics taken from a larger work (*Biology*, 5th Edn; for review see p.128). I imagine there may be many first-year students who would enjoy the engagingly direct, almost conversational text. Some of the illustrations (of an Ames test, a sequencing gel, a DNA clone) score hits, and this seems a good place to meet the immune system. The superb cover illustration of cytoskeletal filaments is rather misleading, however, because this area is thinly covered (organelles as such occupy only 20 pages), and there is no account of

the methods or the microscope which made that image possible. Treatment of macromolecules is also weak. For example, the account of protein structure seems to suggest that the distribution of hydrophilic versus hydrophobic residues accommodates well with the conformation, rather than helps to engender it. Glycosylated membrane proteins are represented as exclusively extrinsic.

Berns's (smaller) book is best where his own technical interests enliven descriptions of methods of study of cells. Overall, however, unevenness of authority is a serious problem. For example, the new account of cell-cell junctions is unfortunately confused about the function of tight junctions. Actin bundles simply are not found "along the leading edge" of migrating tissue-culture cells. In view of the author's background, the discussion of membrane fluidity is surprisingly weak too. Rather giving the game away is the statement that the great interest of viruses to biologists is their involvement in disease and use in genetic engineering.

I promised myself I would actually buy the first textbook of cell biology which in discussing cellular differentiation gave proper prominence to clonal inheritance. Besides a whiff in Kimball's discussion of the immune system, you have to go to the committee volume for that, as you do for a satisfactory balance between what cells can do and the bits and pieces inside which make it all possible.

In that it deals only with experimental work, Bregman's book stands apart from the other three. His manual shows how he has been able to make a variety of biological phenomena accessible to students limited to three-hour spells in the laboratory. The experiments range from the run-of-the-mill to some such as flagellar regeneration in *Chlamydomonas* and DNA replication in leukocytes (using Hoechst 33258 without a fluorescence microscope) which are more of a surprise and therefore especially welcome. Between clearly written background introductions, and detailed inventories of requirements, lie experimental procedures that certainly appear to be robust enough to work after transplantation from the author's own teaching-laboratory.

To judge by the inclusion of data-report pages and printed graph paper, the publishers clearly hope the manual will be bought as a bench book for use by individual students. I suspect that most teachers with the resources (such as microscopes and spectrophotometers) to mount these experiments probably assemble their own laboratory courses by picking and choosing. Many will want students to start to handle radioisotopes and to grow cells other than leukocytes. I am afraid they will go to Bregman just for his best ideas. □

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Beyond Fawcett

R.P. Gould

Histology: Cell and Tissue Biology, 5th Edn.

Edited by Leon Weiss.

Macmillan Press, London/Elsevier, New York: 1983. Pp.1,219. £30, \$49.50.

Cells and Tissues: An Introduction to Histology and Cell Biology.

By Andrew W. Rogers.

Academic: 1983. Pp.242. Pbk £12.50, \$23.

Multiple Choice Questions in Histology.

By Raymond Coleman.

Pitman, London/Urban &

Schwarzenberg, Baltimore: 1983. Pp.285. Pbk £5.95, \$10.50.

HISTOLOGY textbooks come in all sorts of sizes and formats and a matching range of prices. Two of the books under review represent the upper and middle parts of the choice of texts currently available to students. The third is rather different, being a compendium of multiple choice questions on histology.

Histology: Cell and Tissue Biology, edited by Leon Weiss, is the fifth edition of a book first published in 1966 under the editorship of Roy Greep. It is one of the three great books on histology written in English — the others are Ham's *Histology* and Fawcett and Bloom's *Textbook of Histology* — and is found in most university libraries and departments of anatomy. Fawcett and Bloom (the tenth edition of the original Maximow and Bloom) appeared in 1975 and was the work of Don Fawcett. It is still a splendid book, well written, accurate and beautifully illustrated; it shows all the hallmarks of its author's approach to the subject, being scholarly, meticulous, balanced and revealing Fawcett's marvellous appreciation of the visual appeal of the subject. It is perhaps not surprising that at least five of the contributors to Weiss's volume were at some time working in the Department of Anatomy at Harvard when Fawcett was chairman there.

Fawcett's book exhibits an engaging uniformity of style expected of a single author. However, it does not contain that relation of function to form so essential in a modern

Textbook supplement — prices

Where possible both dollar prices pertaining in the United States and sterling prices for the United Kingdom are given in the bibliographical details for each book. Export prices will generally be higher. If a dollar or sterling price is not cited, the book is not available in one of the two countries. However readers should check both price and availability of books before ordering.

An index to all of the books reviewed in this issue appears on pp.143–144.

histology text. This is only to be expected. The exponential growth during the past 30 years of our knowledge of cells, tissues and organs makes it an almost impossible task for a single author, except in a patchy way, to cover in an advanced text the whole of functional histology. As in many other areas of the biosciences, the answer is a multi-author book.

The new edition of Weiss is a complete justification of this approach. Each chapter has been written by an expert and is in essence a "minomonograph" on the subject under consideration. Weiss has not imposed uniformity on the structure of each chapter, particularly those dealing with the organs, so the emphasis placed on the functional aspects varies considerably, ranging from molecular biology to physiology. Many new topics have been introduced — for example the enteroendocrine system — with the recognition of their importance in our understanding of the pathology of those systems. The illustrations, particularly the electron micrographs, are on the whole first-class, but here and there some of the older figures of human material no longer match up to the standard of the rest. In all, however, the new Weiss is a splendid achievement.

As its subtitle declares, Andrew Rogers's *Cells and Tissues* is an introduction to histology and cell biology. In the first half of the book the author covers structure, with useful emphasis on how to look at and interpret the architecture of a conventionally stained histological section. In the second half, Rogers then pursues a number of general questions about the histology of the body — how the tubes of the body are constructed and how the different parts of the body communicate with each other, for example. Here the author ranges over the whole body; thus, all the tubes from the different organ systems are compared and in the second chapter of this section there is a mixture of information about both the nervous and the hormonal systems. The final chapter deals with the interpretation of abnormal structure.

One gets the feeling that the book is based on a course of lectures in which an attempt was made to avoid the usual systematic approach to the subject. It is a brave try, but does not, I feel, quite come off.

Raymond Coleman's book of MCQs in histology will prove to be a valuable source of such questions for tests and examinations using this form of assessment. In particular, the fairly full explanatory notes to the answers given will undoubtedly help the examinee. One can only hope, however, that this relatively easy if useful way of testing a student's knowledge will not completely replace either essay-type questions or the examination of real histological specimens down a microscope. □

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Interpretations of biochemistry . . .

E.D. Saggerson

Principles of Biochemistry, 7th Edn, in two volumes.

By Emil L. Smith, Robert L. Hill, I. Robert Lehman, Robert J. Lefkowitz, Philip Handler and Abraham White.

McGraw-Hill: 1983. General Aspects pp. 886. Hbk \$36, £29.95; pbk £10.95. Mammalian Biochemistry pp. 759. Hbk \$42, £34.95; pbk £10.95.

Biochemistry.

Senior author Geoffrey Zubay. *Addison-Wesley: 1983. Pp. 1,268. \$39.95, £18.75.*

Biochemistry, 2nd Edn.

By Frank B. Armstrong. *Oxford University Press: 1983. Pp. 653. Hbk \$27.95, £24.50; pbk £9.95.*

THOSE who were familiar with the sixth edition of *Principles of Biochemistry* will recognize many of the figures in this new edition, and also certain features such as the sectionalization. They will, however, find that much else has changed.

The seventh edition comprises two distinct but related volumes (*General Aspects* and *Mammalian Biochemistry*) and the format has been improved enormously. In *General Aspects* red has been adopted as a second colour for use in figures and headings so that the overall impression is of a book which is now more like those of Lehninger and Stryer. All sections show signs of considerable reworking, updating and expansion being most evident in the accounts of hormone receptors and hormone action, molecular genetics and biochemistry of the immune system. The metabolism section which was really rather dull in the previous edition is now presented in far more attractive way.

Principles of Biochemistry remains the Rolls Royce of biochemistry textbooks — both in terms of price and overall scope. Containing approximately 25% more material than its predecessor, this new edition is a formidable collection of biochemical knowledge with its primary emphasis still being on mammalian biochemistry. A general student might be satisfied with just *General Aspects* (structures of biomolecules, enzymes, metabolism, molecular genetics) but students in the medical sciences would obviously have to progress on to *Mammalian Biochemistry* (body fluids, endocrine system, specialized tissues such as nerve, muscle, eye, gut and human nutrition). Indexing of the books is excellent (taken together the two volumes have 120 pages of index). Referencing, too, is good, each chapter being followed by lists of books and reviews (up to 1982), though unfortunately there is considerable

cross-referencing between the two volumes which could be annoying for a reader who has only one of them.

Any newcomer to the jostling throng of general biochemistry textbooks is likely to go unnoticed unless it really has something extra to offer. While Zubay's *Biochemistry* is an adequate general book covering the subject at a level similar to Stryer or Lehninger, I am not convinced that it will make a major impact. The 32 chapters are written by 26 different contributors, which leads one to hope that the book would be really up to date; so it is disappointing to find that none of the rather few selected texts listed at the end of each chapter is more recent than 1981.

On the merit side, *Biochemistry* contains some excellent figures — in black and red colouring — particularly in the sections on protein structure. There is also a fascinating final chapter on the origins of life. On the other hand, although metabolism is adequately covered there is little attempt to integrate it or to tackle the control of metabolism. Reading the section on the control of glycogen metabolism one would have thought that nothing had happened in this complex and active field since about 1970 — compare this with the up-to-date and

complex presentation in *Principles of Biochemistry*. Other examples of where more attention was needed are the very scant descriptions of biologically important proteins such as collagen or the immunoglobulins.

The second edition of *Biochemistry* by Armstrong is a reworking of the book of the same title by Armstrong and Bennett which appeared in 1979. In a previous review (*Nature* 283, 902; 1980) I wrote of this book that "the text is clearly and concisely presented and the figures are good". I have no reason to change my mind on reading the new edition. The book is approximately 30% longer than its predecessor, the increase in extent being mainly due to the inclusion of a new chapter on lipid biosynthesis — a glaring omission from the first edition — and the addition of a chapter on recombinant DNA research. Each chapter is followed by a sizeable list of further readings (both books and articles) and interesting problems and questions. *Biochemistry* has much in its favour as a general introductory text. □

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. . . and of its human alternatives

Thomas A. Scott

Human Biochemistry.

By Wilhelm R. Frisell. *Macmillan, New York/Collier Macmillan, London: 1983. Pp. 845. \$37.50, £31.75.*

Essentials of Human Biochemistry.

By C.R. Paterson. *Pitman, London/Urban & Schwarzenberg, Baltimore: 1983. Pp. 275. Pbk £8.95, \$15.50.*

Biochemistry for the Medical Sciences.

By E.A. Newsholme and A.R. Leech. *Wiley: 1984. Pp. 951. Hbk £49, \$99; pbk £19.75, \$39.95.*

THE boundaries of disciplines within the life sciences are determined to a large extent by the judgement (or caprice) of individual authors and designers of courses. No subject demonstrates this more vividly than human biochemistry. Indeed the very inability to agree on a name (physiological chemistry, medical biochemistry and molecular physiology are just a few alternatives) suggests a crisis of identity. Thus, Dr Frisell prefers to base himself uncompromisingly in pure biochemistry with occasional excursions of physiological relevance, whereas the other authors attempt a total integration of medicine and metabolism.

Human Biochemistry by Frisell is a large

textbook of over 800 pages, resembling at first sight some of the encyclopaedic treatises that have appeared during the past decade. Closer inspection reveals a sound presentation of basic biochemistry with a physiological bias, intended for a single semester course. Possibly this is the reason why some topics, membrane structure and chemiosmosis for example, are absent or treated dismissively.

Part One ("Basic Biochemistry") follows a conventional pattern based on the structure and metabolism of the chief classes of biochemical compounds, together with enzymology and bioenergetics. Part Two ("Metabolic Basis of Human Biochemistry") discusses higher levels of organization, thereby emphasizing the physiological perspective of the basic science presented in Part One. Dr Frisell might have given his book more stature by integrating the biochemistry with the pathology of disease, but he apparently sees the cut-off point between these two disciplines as lying very close to fundamental biochemistry. Even in the lengthy chapter on the elements of nutrition, prominence is given to the listed dietary requirements and metabolic roles of food components, with hardly any discussion of the chemical-pathological basis of deficiency diseases. The treatment of prostaglandins and the biochemistry of nerve tissue sparkle with a certain freshness, but in general the style of presentation is familiar and orthodox.

The author is obviously a teacher of considerable experience, and he never loses sight of his most important reader, the undergraduate student. Every chapter

concludes with a section entitled "Problems and Clinical Correlation", with answers at the end of the book. These well-chosen questions orientate the student towards the key facts and reasoning of the preceding chapter. There is also a useful glossary of biochemical terms, and a very useful table of constituents of body fluids with their normal ranges.

Dr Frisell's book can be recommended to both medical and non-medical students, but they would probably prefer to find it in the library, rather than buy a personal copy.

In contrast, Paterson's *Essentials of Human Biochemistry* is small, reasonably priced and exciting. The description of biochemical pathways is totally integrated with the discussion of clinical disorders, amply illustrated with photographs and informative diagrams. No space is wasted in an economic juxtaposition of metabolic schemes, pictures and text, with the result that the book contains a surprising amount of information for its size. I was just a little disappointed in the chapter on hormones; there is insufficient stress on the central role of the pituitary, and I think the catechol oestrogens now deserve to be mentioned in teaching texts.

Dr Paterson has taken advice from a large number of experts in various areas of medical biochemistry, so that the text has an air of authority and is very up to date. This book can be strongly recommended for medical students and all biochemists with an interest in the medical aspects of their subject.

A senior colleague once jokingly suggested that the secret of impressive lecturing was to use an erudite text and not tell the students about it. *Biochemistry for the Medical Sciences* was surely the kind of book he had in mind. In this individualistic treatment of human biochemistry, structural and molecular aspects are excluded, and the approach is frankly metabolic. The usual pathways of intermediary metabolism are described and discussed in detail, and the treatment of energy metabolism and thermodynamics is particularly useful and is well geared to the general requirements of most students of medicine.

Above all, however, this book is characterized by its devotion to the integration of clinical physiology and biochemistry with a powerful emphasis on control and regulation. The tutor in search of a theme will be inspired by such titles as "Pathological changes in concentrations of lipid fuels" and "Metabolic responses to starvation", and by a delightful section on the comparative biochemistry of sprinting and marathon running. Within a rather novel framework the authors have produced a work of scholarship, which all lecturers should read and no student can afford to ignore. □

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Matters physical and chemical

E.G. Richards

Physical Biochemistry: Applications to Biochemistry and Molecular Biology, 2nd Edn.

By David Freifelder.

W.H. Freeman: 1982. Pp.761.

Hbk \$43.95; pbk \$24.95, £21.50.

Analytical Biochemistry.

By David J. Holme and Hazel Peck.

Longman: 1983. Pp.480. £26, \$57.

THE physical methods used in biochemistry and molecular biology appear all too complicated to the student with little physics or mathematics, and one has come to expect such students to be daunted by books that deal with the principles of physical biochemistry in all their physical chemical detail. It is a rare textbook that does not have this effect. The second edition of *Physical Biochemistry* is one.

Freifelder has performed the notable service of bringing a large number of techniques and concepts within the comprehension of undergraduate and graduate students who are not primarily of a physical bent. While quite a number of useful formulae are scattered through the text, not many of them are derived and there is little mathematics. Instead, the emphasis is on qualitative and intuitive understanding. The text is lucid, the examples apposite and the diagrams up to the usual standards of W. H. Freeman. This book should help students to understand matters physical and help to dispel some of their mystery.

Physical biochemistry is defined liberally to include light and electron microscopy, immunological methods and DNA sequencing, as well as the more traditional techniques of ultracentrifugation, spectroscopy and fluorimetry — to mention but a few of an encyclopaedic range of topics covered (though there is nothing on crystallography). There are many detailed and illuminating analyses of real applications and each chapter includes a reading list and a set of problems (the answers being provided at the end of the book).

The original edition of this book was marred by a few unfortunate mistakes and some notable omissions. The mistakes have been corrected and the text expanded to include light-scattering techniques (among several other new subjects) and fuller descriptions of some topics already in the first edition. This second edition deserves to be popular with both students and their teachers. My only caveat is that graduate students will require something more meaty. The reading lists provided should set them on the right path to this.

It may be argued that chemical analysis is the *sine qua non* of serious biochemistry.

Certainly, specialized techniques are required, and in *Analytical Biochemistry* Holme and Peck have provided a useful compendium of such methods.

The book comes in three sections, in turn covering analytical methods, useful materials for analyses (enzymes, radioisotopes, antibodies), and the analysis of major groups of biologically important compounds (carbohydrates, amino acids, proteins and lipids — though, oddly, there is little on nucleic acids). The methods considered include spectroscopic, electro-analytical and immunological approaches in most of their various forms, as well as those which involve the use of radioisotopes and separation techniques. Both the theoretical background and the instrumentation involved are discussed succinctly, in adequate though not too exhaustive detail, and each chapter includes a list of further reading. The statistical aspects of the subject — perhaps given too little emphasis in some parts of the biochemical literature — are here properly discussed and duly emphasized.

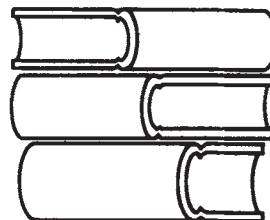
It is useful, as the authors suggest, to have all these methods housed in one volume and *Analytical Biochemistry* will remain on my bookshelf as a source book. It deserves a similar fate with both undergraduate and graduate students. □

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Photosynthesis: some light relief

Peter D. Moore

C₃, C₄: Mechanisms, and Cellular and Environmental Regulation, of Photosynthesis.

By Gerry Edwards and David Walker.
Blackwell Scientific/University of California Press: 1983. Pp.542. £32, \$65.

Photosynthetic Systems: Structure, Function and Assembly.

By Susan M. Danks, E. Hilary Evans and Peter A. Whittaker.
Wiley: 1983. Pp.162. Hbk £13.95, \$26.25; pbk £5.95, \$11.50.

AS ANY purveyor of academic materials can tell you, one of the most important components of a good lesson or lecture is an element of humour. It can turn an adequate and accurate presentation of information into a memorable one. It is surprising, therefore, that so few scientific textbook writers are prepared to inject humour into the printed page. The joke or pun which is cheerfully delivered from the rostrum is usually omitted from the written version, which often loses vitality as a result. Gerry Edwards and David Walker have thrown caution to the winds and have included jokes, even cartoons, in their book *C₃, C₄*, and the result is not only amusing, it is memorable.

The subject in this case is photosynthesis and the treatment it receives here will prove both informative and stimulating to the novice and the research worker alike. It begins with a lucid account of thermodynamics and energetics (where we are told, among other things, that Joules are a girl's best friend) and develops, through the biochemistry of pigments, to a consideration of the "Z" scheme and ATP formation. Carbon fixation pathways are then described and explained, with the help of intermittent light relief, such as "Up the Carbon Path" — a story of cellular espionage. It may not add much to the science, but it contributes greatly to the readability of the book.

Much space, as the title indicates, is given over to a description of the *C₄* pathway and its comparison with the conventional *C₃* and CAM strategies. This is not an easy task but the authors conduct it in a clear and flowing fashion. Wherever a simple statement can be made in an unambiguous manner, it is done so. Where questions remain unresolved, at least they are set out clearly, and periodic summary statements provide useful milestones in the development of their theme.

The only areas in which the book deserves criticism are its length, which makes it an unlikely candidate for the undergraduate market to which it is otherwise so admirably suited, and its type-

setting, which has left such extensive, naked lacunae at the top of each page that the text often pushes the page numbers off the bottom.

The second book reviewed here, *Photosynthetic Systems*, is more compact than *C₃, C₄* but covers much of the same ground. There is a greater emphasis on structure and a more liberal use of electron micrographs. There is also more stress on the evolution of photosynthetic systems, but less information about the historical aspects of the formulation of current theories, and less evidence of humour.

Again, the approach is simple and clear. Even fairly complex areas such as ATP synthesis have the questions which need answering framed in a simple yet logical fashion. *C₄* and CAM mechanisms are dealt with rather briefly, but a major section is included on plastid development

and chloroplast genetics.

Comparing the two books, there is a clear difference in emphasis, Edwards and Walker concentrating more upon *C₄* and CAM and their relative physiological and ecological advantages, Danks, Evans and Whittaker taking more excursions into related subjects, such as nitrogen fixation, biosynthesis of pigments and chloroplast DNA. *Photosynthetic Systems* is also more precisely aimed at the undergraduate, whereas *C₃, C₄* starts at a lower level and proceeds to a higher one. Which is the preferable book will therefore depend very strongly upon the nature of its proposed use. Personally, I would go for *C₃, C₄* — and not just for the laughs. □

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Working plants

Michael Black

Plant Physiology, 4th Edn.

By Robert M. Devlin and Francis H. Witham.

Willard Grant Press/Wadsworth: 1983. Pp.577. \$27.

Introductory Plant Physiology, 2nd Edn.

By G. Ray Noggle and George J. Fritz.

Prentice-Hall: 1983. Pp.627.

\$45.85, £32.25.

Biophysical Plant Physiology and Ecology.

By Park S. Nobel.

W.H. Freeman: 1983. Pp.608.

\$36.95, £34.95.

Plant Physiology in Relation to Horticulture, 2nd Edn.

By J.K.A. Bleasdale.

Macmillan Press, London: 1984. Pp.143.

Pbk £5.95.

PLANT function increasingly is being understood in formal chemical and physical terms. Biochemists continue to elucidate aspects of plant metabolism that were hitherto poorly understood, molecular biologists are beginning to provide some understanding of the control of differentiation and morphogenesis, and some aspects of ecophysiology are amenable to analysis in physical terms. Plant physiology is also a subject of great practical importance: manipulation of the growth and metabolism of plant cells, tissues and organs in culture is being exploited, and together with the application of molecular biological techniques carries great promise for agriculture and biochemical engineering. A textbook of plant physiology should, of course, reflect these trends. To varying degrees, each of these four books meets the requirement.

Devlin and Witham's *Plant Physiology* (now in its fourth edition) and Noggle and Fritz's *Introductory Plant Physiology* (in

its second) concentrate on higher plants and cover essentially the same ground, at a comparable level, but with slight differences in emphasis. Both start with concise accounts of cell structure and physiology, but then diverge — *Introductory Plant Physiology* into metabolic physiology and then diffusion, transport phenomena and mineral nutrition, with *Plant Physiology* reversing the order of treatment. Both books offer highly readable and competent reviews of these subjects but each with some advantages over the other. Devlin and Witham, for example, include separate chapters on proteins and nucleic acids, enzymes and carbohydrate metabolism, whereas these topics are compressed into one chapter by Noggle and Fritz. In *Plant Physiology* carbon dioxide fixation is separated from electron transport and photophosphorylation, but these are all covered together in *Introductory Plant Physiology*. The former treatment is likely to be clearer for students. On the other hand, photorespiration receives special consideration by Noggle and Fritz, who give a good, comprehensive account of the phenomenon. And to their credit, both books nicely set this topic, together with *C₃* and *C₄* photosynthesis, in the context of plant productivity and agriculture.

The last third of each book is devoted to the physiology of growth and development, plant growth substances occupying a prominent role. The treatments are generally good, but conventional, for neither transmits much of the growing debate in this field (for example over long-cherished concepts such as the Cholodny-Went theory of tropic movements). Slight deficiencies can be found in other respects too. The discussion of phytochrome is consigned by Noggle and Fritz to an early chapter on photophysiology, where it joins the photochemistry of chlorophyll, carotenoids etc., and the authors are unsuccessful in bringing the topic back to where it really belongs — with the control of extension growth, flowering, germin-

ation and other aspects of photomorphogenesis. Neither book conveys the importance of phytochrome in an ecological context, even though so much has been learned about this in recent years.

Devlin and Witham may just have the edge as far as the student is concerned, for in their book the diagrams and photographs are rather better integrated with the text; nonetheless, *Plant Physiology* sometimes falls behind in the depth and "up-to-dateness" of its coverage. But while any attempt to deal with such a broad area is bound to be open to criticism, I would be happy to recommend either of these books. Students of biological science who are likely to meet plant physiology at any time in their university career can be directed to both works with confidence.

Nobel's book, which follows his earlier *Biophysical Plant Physiology*, published by Freeman in 1974, is not comparable with the preceding two works, for it deals only with those parts of plant function which are amenable to physicochemical analysis — ion and water movement, energy transduction and bioenergetics, temperature-energy budgets, gaseous fluxes in leaves, and aspects of photoperception. In the author's words, the book is "an attempt to integrate at an introductory level, certain relevant parts of the physical sciences with plant biology". It succeeds in this aim. Treatment is at a depth not found in any other plant physiology textbook of which I am aware, yet Nobel assumes no more than a first-year university background in physics, chemistry and mathematics on the part of his readers. All interested in eco-physiology, transport phenomena and bioenergetics will find this an invaluable if not essential companion to books dealing with these subjects in a more conventional manner, and plant physiologists in general will appreciate the appendices on the symbols, abbreviations, physical constants, co-efficients and conversion factors with which we are having to become familiar.

Bleasdale's *Plant Physiology in Relation to Horticulture* is intended for a different readership, primarily for horticulturists. It gives an elementary account of those aspects of plant function about which such practitioners should be aware, but it would make useful reading for biologists who wish to know something about the practical importance of plant physiology. The book is a revised version of the first edition (1973) and the author admits to surprise at how little needed to be changed. But I wish that he hadn't been so conservative, at least with the references. For example, the average age of the citations on photosynthesis (in the chapter on seedling physiology) is 37 years, and even the "new" chapter on the seed mostly refers to material nearly 20 years old. □

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Past attractions

Keith Allen

Paleobotany and the Evolution of Plants.

By Wilson N. Stewart.

Cambridge University Press: 1983.

Pp. 405. £17.50, \$29.95.

Introduction to Palaeobiology: General Palaeontology.

By B. Ziegler.

Ellis Horwood/Halsted: 1983. Pp. 225.

Hbk £30, \$69.95; pbk £12.50, \$29.95.

Ancient Sedimentary Environments and the Habitats of Living Organisms: Introduction to Palaeoecology.

By Jean-Claude Gall.

Springer-Verlag: 1983. Pp. 219. DM 60, \$24.80.

A TEXTBOOK can be compared with a member of the opposite sex. If the initial layout is attractive, then you may wish to continue the acquaintance. *Paleobotany and the Evolution of Plants* by W.N. Stewart is a good-looking book and repays closer attention. The text is well laid out, the photographs well produced, and the new and redrawn figures are of high quality (no acknowledgement is given for these, so I assume they were drawn by Stewart himself).

After a gap of almost twenty years (1961–1981) when no general British or North American palaeobotany textbook was published, Wilson's book follows closely on the heels of Thomas Taylor's *Paleobotany: An Introduction to Fossil Plant Biology* (for review see *Nature* 295, 468; 1982). Both texts are aimed at a similar audience, primarily the palaeobotanist and botanist, with little for the geologist. Stewart's chapters follow the usual sequence — starting with types of plant preservation and techniques for their study, continuing with classification, and then running through the taxa from cyanobacteria to angiosperms.

This textbook is well written and well researched. In all, it is a pleasure to own. My main criticism is that the angiosperms, which have after all dominated the Earth's flora since Upper Cretaceous times, are given only 27 pages in a book of 400. This is a pity; at present there is a lot of interesting research being carried out on early angiosperms which might have been mentioned.

Although the task of giving an overview of palaeobiology in 225 pages seems a daunting one, my initial impression of

Textbook supplement — prices

Where possible both dollar prices pertaining in the United States and sterling prices for the United Kingdom are given in the bibliographical details for each book. Export prices will generally be higher. If a dollar or sterling price is not cited, the book is not available in one of the two countries. However readers should check both price and availability of books before ordering.

Ziegler's *Introduction to Palaeobiology* was favourable. It is well laid out, and is accompanied by pleasing illustrations. Here I thought is a book to interest biologists and geologists alike. Sadly, on delving deeper, my initial enthusiasm turned to disappointment.

The book was originally published in German in 1972; it is in part out of date and has not been thoroughly researched. In Chapter 1, for instance, "Major Sub-divisions of the Organic World", errors are woefully abundant. We are told that living things can be divided into two kingdoms, then four are listed. Phyla are used in the Plant Kingdom when, under the Rules of the Botanical Code, Divisions are mandatory. The Mastigophora are included in both the Plant and Animal Kingdoms; what is wrong with the Kingdom Protista for the unicellular algae and protozoa? The name Phycomycetes, used here, became "extinct" in the early 1960s, while according to Ziegler *Rhynia*, *Lepidodendron* and *Equisetum* are ferns and gymnosperms are flowering plants. There are many other inaccuracies in this chapter and it is all very confusing for students.

Topics covered in other chapters include evolution, mode of life, ecology and biogeography. In some there are further serious errors and the reference lists for each chapter are too short and inadequate. There are other, better books on the subject, for example D. M. Raup and S. M. Stanley's *Principles of Paleontology* (W. H. Freeman, 2nd Edn 1978), which students would be advised to turn to first.

Gall's *Ancient Sedimentary Environments and the Habitats of Living Organisms* looks less attractive. Unkindly you might say that in appearance it is a book of prominent headings interspersed with text. Nevertheless the more I read this book, the more I liked it. It is in two parts, the first giving information deduced from the fossils and sediments (e.g. modes of life, sediments and sedimentary environments and fossiliferous horizons), the second dealing with nine ancient sedimentary environments of differing types, from the Precambrian Ediacaran fauna to an Acheulian hunter's cave. It includes discussion of various marine, lagoonal, deltaic and fluvial environments.

It seems to me, however, that this book is too short; a further 50 pages would have allowed greater depth and the elucidation of somewhat telegraphic statements. It was written in 1975, and does not appear to have been updated for translation — only four references are given from the past decade — while a palaeobotanist could have advised Gall on several errors. But this is a generally good elementary text, which could be of use at first-year university level, particularly for biologists needing some relevant geology. □

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Grades of evolution

Paul H. Harvey

Evolutionary Biology. By Eli C. Minkoff.
Addison-Wesley: 1983. Pp.627.

\$29.95, £21.70.

Sex, Evolution, and Behavior, 2nd Edn.

By Martin Daly and Margo Wilson.

Willard Grant Press/Wadsworth: 1983.

Pp.402. \$17.55.

Natural Selection and its Constraints.

By Oliver Mayo.

Academic: 1983. Pp.145. Hbk £12,

\$19.50; pbk £5.95, \$9.50.

ENCYCLOPAEDIC textbooks are expected to be a little boring. They also, inevitably, introduce and perpetuate a few errors. But Eli C. Minkoff has managed to purvey so much erroneous information in a book that surveys the whole field of evolutionary biology that I could not possibly recommend it as a course text. Several old favourites, such as character displacement in nuthatches and the surface area interpretation of Bergmann's Rule, are recreated for the misinformation of a new generation of students. Those same students will attempt to understand why females of haplodiploid species share 25% of their genotypes with their sons. And they will even be told that there is good evidence that the sex ratio responds to selection in *Drosophila*. I could go on. Fortunately, and contrary to the author's claims, there is a textbook that covers accurately most of the same ground in evolutionary biology. It has the same title as Minkoff's but was written by Douglas Futuyma and published by Sinauer in 1979.

It is a pleasure to turn from an unsatisfactory book to a good one. Daly and Wilson's *Sex, Evolution, and Behavior* has now appeared in a second edition which is even better than the first. Since 1978, when their first edition was published, a lot has happened in sociobiology. Many adaptive stories, particularly relating to human behaviour, have been subjected to comparative and experimental tests. Eric Charnov has brought together a disparate series of research topics, concerned with how individuals partition resources into male and female function, into a unified theoretical discipline which he calls sex allocation theory. These and other developments are integrated by Daly and Wilson into a wholly rewritten text.

There are a few mistakes, and I was particularly unhappy about their treatment of some aspects of sexual selection — Fisher did *not* model the runaway process and Zahavi's handicap principle is *not* likely to explain peacocks' tails, even when "genetic modelers approach [it] in the right spirit". Such flippancy treatment of formal mathematical arguments characterizes a lot of work in this area and has led to several disagreements caused by loose

thought. Indeed, Daly and Wilson's discussion of "confidence of paternity" confused me precisely because they did not give a formal presentation of their verbal argument; I do not know what assumptions lead to their conclusions. The book carefully avoids mathematical arguments, presumably because much of the intended audience (which includes social scientists) prefers the intuitive insights that can accompany verbal presentation.

Mayo's useful little book lies at the other extreme. He presents his audience with a terse series of eleven essays that attempt to survey the constraints within which natural selection works. Topics covered include allometry, the rate of evolution, canalization, senescence and speciation. This is not a book for undergraduate study, nor does it attempt to explain concepts or to review fields of research. For example, senescence is allotted two pages. Instead, Mayo provides a framework within which graduate students can study contemporary problems in evolutionary biology. Key papers up to 1981 are cited in each section, together with a series of topics for discussion. This makes excellent material for a series of graduate seminars in evolution and I intend to use the book for exactly that purpose. □

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Anatomical types

T.S. Kemp

Comparative Anatomy of the Vertebrates, 5th Edn.

By George C. Kent.

Mosby/Blackwell Scientific: 1983.

Pp.604. \$29.95, £26.

Analysis of Vertebrate Structure, 2nd Edn.

By Milton Hildebrand.

Wiley: 1983. Pp.654. Hbk £30.35, \$42.50;

pbk £5.95, \$11.50.

COMPARATIVE anatomy of the vertebrates is continuing to flourish, it seems, judged by the appearance of a fifth edition of George Kent's *Comparative Anatomy of the Vertebrates*. In this age of theory, principles and problem-solving in biology, one wonders exactly what is the role of such a descriptive book. True, lip service is paid to functional anatomy, adaptation, phylogeny and evolution, and occasionally development, but the great bulk of the book is straightforward description, modernized in details but indistinguishable in approach from the equivalent works of fifty years ago.

What Kent fails to convey is the great strides in methodology and philosophy that have underwritten the various aspects

of comparative biology. In the book classification and phylogeny are treated as virtually synonymous which is by no means necessarily the case; functional interpretation is restricted to comments on single features, despite the growing holistic view of animal function; and the various concepts of morphology are considered no more worthy of theoretical treatment than pictures in a dissection guide. But Kent's book is no worse than several others in the field, and at least he hints here and there that the subject might have more depth than he makes explicit.

The question is, then, whether there is a place in teaching for a volume such as this. The answer depends on the use to which the book is put. If it is meant to provide a self-contained course of study, then it is not a course that I should wish to give to undergraduates. As a reference source, a kind of glorified handout, accompanying an interesting course on the biology and evolution of the vertebrates, then it does have merit. After the obligatory chapters on the characters and origin of the vertebrates, there is an extremely superficial who's-who of the group. There is then a series of chapters, quite unrelated to one another, covering the various classic vertebrate systems one at a time. Plenty of good-quality illustrations, often made clearer by the use of red and blue colouring, are included. Each chapter has a reference/literature list which tends to be rather idiosyncratic and on the whole rather out of date. This is a dull, basically worthy textbook.

The second edition of Milton Hildebrand's *Analysis of Vertebrate Structure* is much more welcome since his explicit aim is to show how structure is related to function, in which he succeeds well. After a brief outline of vertebrate history, there is a long system-by-system comparative section, but in this case the adaptive and functional meanings are clearly brought out. The anatomy is thus made to work for its living, so to speak, generating a lively account. The best part of the book is the last section, which is a clear and quite detailed exposition of the various forms of vertebrate locomotion, showing how the skeleton and muscles are designed to perform the required functions efficiently. A final part treats feeding mechanisms in vertebrates from the same point of view.

I have found the first edition of Hildebrand a valuable teaching text for undergraduates, complete with its excellent illustrations and useful reference lists. This edition continues the tradition, and has been brought up to date by the addition of new references and a number of new diagrams. In the words of the preface "Finally, it is hoped that this book will be found interesting". It is. □

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Variation in good behaviour

Ian J. Patterson

Animal Behaviour: Ecology and Evolution.

By C.J. Barnard.

Croom Helm/Wiley: 1983. Pp.339.

Hbk £17.95; pbk £8.95, \$21.50.

The Study of Animal Behaviour.

By Felicity Huntingford.

Chapman & Hall: 1984. Pp.411.

Hbk £24.50, \$49.95; pbk £10.95, \$22.

THE many texts on animal behaviour published over the past ten years have increasingly reflected the growth of interest in functional and evolutionary aspects of the subject. In particular, the emphasis on individual genetic fitness, gene selection and the strategies which animals should pursue to maximize fitness has culminated in a number of specialist books on socio-biology and behavioural ecology.

C.J. Barnard draws back from this extreme. His title is well-chosen in that the first half of the book covers straightforward ethology (the physical basis for behaviour, external and internal causal factors, with genetic and environmental influences on development), while the second deals with behavioural ecology and evolution (habitat selection, foraging theory, anti-predator behaviour, mating strategies and social behaviour). A final chapter draws together the second half of the book through a discussion of the evolution of behaviour, including a rather puzzling return to kin selection and evolutionarily stable strategies which are covered previously in Chapter 3.

The text is clearly written and easy to follow, although some rather complex examples (for instance the account of newt courtship on p.70) lack a sufficiently full explanation for readers not familiar with the original work. The lettered keys on figures are tedious to read and sometimes it is unreasonably difficult to extract information from them; on p.91, for example, the text must be related to letters in the figure through a translation in the legend. The references too would have profited from greater care. Some authors are cited by name, others by a superscript reference number, while a few authors named in the text do not appear in the reference list or lack the usual superscript. Also, pertinent information sometimes appears without any reference at all (in Section 1.2.1. for example). Such sloppy presentation will frustrate the serious student anxious to follow up examples in detail.

These minor problems, however, do not detract seriously from what is an excellent and well-written undergraduate text. *Animal Behaviour* gives a good overview of the current state of play in this discipline

and will provide a firm basis for later specialization.

In *The Study of Animal Behaviour* Felicity Huntingford has covered a similar range of subject matter in a rather more conventional way than Barnard, with behavioural ecology being confined largely to one very long chapter on the adaptive significance of behaviour (taking up over a third of the book). The remainder provides fairly advanced coverage of ethology, at a level suitable for senior undergraduates. The figures and tables are clear and easy to assimilate and the writing only rarely drops from a high standard of clarity; some of the exceptions, however, are spectacular (for instance the penultimate sentence of Section 5.8.5e on p.229).

Perhaps the most radical aspect of

Huntingford's approach is the omission of the customary chapters on neuroanatomy and physiology and the structure and function of sense organs. It can be argued that knowledge of such "hardware" is not essential to an understanding of ethology, but most lecturers nevertheless include at least some coverage of the physical basis of behaviour in their courses and so may be reluctant to use this book as a sole text. Included in it, however, are very useful discussions of the description and measurement of behaviour and applied ethology, which add to the undoubted value of *The Study of Animal Behaviour* for student use. □

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Monkey business

W. C. McGrew &
J. R. Anderson

Primate Social Relationships:

An Integrated Approach.

Edited by Robert A. Hinde.

Blackwell Scientific/Sinauer: 1983.

Pp.384. Hbk £26, \$40;

pbk £13.80, \$21.

Primate Behavior.

Edited by James L. Fobes and

James E. King.

Academic: 1983. Pp.393. \$29, £20.50.

Primate Social Relationships is an exciting book, both in content and in form. In content it is virtually unique for an anthology in that it is unified by a theoretical framework, the aim being to apply the ideas of Professor Hinde and his students at Cambridge to the analysis of social organization. Social life is seen as a hierarchical system of increasing complexity: individuals behave; when they exchange behaviour they interact; accumulated interactions lead to relationships; networks of relationships yield social structure. The key level in the system is the *relationship*, and the analyses provide both proximate and ultimate explanations.

The testing of the ideas has been done largely by research on three types of Old World monkeys: savanna baboons, vervet monkeys and rhesus macaques. The macaques have been subject to most attention, both in small harem groups in captivity and in a free-ranging population in Puerto Rico. Concentration on these polygynous forms is both a help and a hindrance, however, for while their near-uniformity of social organization allows confident, detailed conclusions to be drawn it prevents generalizations to other primates. To some degree, extension to other forms is tackled in the final chapter, but this is something of a hodge-podge and has the appearance of being an afterthought.

The novelty of the book's form lies in the fact that each chapter comprises from two to ten independent contributions. There are 61 such "mini-chapters", averaging about five pages each; about a third are conceptual in nature, the remainder empirical. Mostly these give concise, specific examples of, for instance, the effects of being orphaned, differences between primiparous and multiparous mothers, matriline membership and acquisition of social rank etc. Given that there are 20 contributors in all, the overall degree of clarity is remarkable, though a few sections read like abstracts of whole chapters from PhD theses.

Primate Behavior is a more conventional piece of compilation, consisting of eleven chapters on various topics in behavioural primatology with an acknowledged bias towards laboratory studies. It will therefore be used best in conjunction with other texts which cover ecology and social relations in greater depth. There are two chapters devoted to these areas, but understandably they suffer from having too much to say in too little space, and so such topics as territoriality, the effects of plant secondary compounds on feeding strategies, and infanticide are illustrated only modestly.

If the two contributions on naturalistic behaviour are better seen as appetisers for field studies than as main courses, two chapters by the editors on learning abilities constitute major reviews of experimental research on primate cognitive processes. Starting from simple conditioning, the authors trace developments in discrimination and reversal learning, learning set and delayed response, to more complex types of performance involving concepts of quantity and analogical reasoning. Object permanence, tool-use and cognitive maps are also discussed. An important point made here is that there has been a shift away from searching for single performance indices for categorizing different species according to "intelligence" to trying to understand the strategies which primates apply to

problems and the underlying mechanisms which can be inferred.

The editors draw attention to the constant risk of unwittingly handicapping an animal through the use of inappropriate testing procedures. The same caution applies to studies of sensory abilities, notably vision and audition, which are described in two further chapters. The account of perceptual processes goes some way towards animating the psychophysics, though much recent experimental work on the perception of social stimuli is omitted. The book also includes contributions on ape language studies, primate evolution, early assessment procedures and influences

on behavioural development, and abnormal behaviour, focusing on the experimental study of depression.

Neither *Primate Behavior* or *Primate Social Relationships* is a textbook in the usual sense. But both are aimed at more than research specialists in primatology and should be useful in teaching postgraduates or keen undergraduates. Together they exemplify the new directions in which primatology continues to grow. □

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Keeping pace with biology

J.T. Bonner

Elements of Biological Science, 3rd Edn.
By William T. Keeton and Carol Hardy McFadden.

W. W. Norton: 1983. Pp. 729. \$16.60, £10.95.

Life: The Science of Biology.

By William K. Purves and Gordon H. Orians.

Sinauer/Willard Grant Press/Blackwell Scientific: 1983. Pp. 1,182. \$33.75, £19.50.

Biology, 5th Edn.

By John W. Kimball.

Addison-Wesley: 1983. Pp. 974. \$31.95, £15.95.

Biology, 2nd Edn.

By Donald D. Ritchie and Robert Carola.

Addison-Wesley: 1983. Pp. 644. \$28.95.
The Biolab Book. By Lundy Pentz.
Johns Hopkins University Press: 1983. Pp. 127. \$9.95, £8.50.

EACH year brings new basic biology texts or new editions of established ones. A group from this year's crop is presently sitting on my desk and weighing it down dangerously. The problems of writing a textbook on general biology are quite extraordinary. The successful ones are enormous and encyclopaedic in their coverage; yet despite their appearance of solidity and finality, the subject changes at a reckless pace which forces the need for successively heavier editions. It is true that some books, of which the new edition of *Elements of Biological Science* by Keeton and McFadden is an example, are specifically designed for a short, one-semester course; but while this book is the third edition of a good, condensed version of the

highly successful Keeton's *Biological Science* (for review see *Nature* 289, 714; 1981) it still tips the scales at over four pounds.

Another characteristic of the new books is that they are beautifully illustrated. It is not just that there is an increasing tradition of clear and helpful diagrams and drawings, sometimes printed in more than one colour, but also of including stunning photographs. I suspect that the great success of *Scientific American* and their pioneering of the art form of scientific illustration has done much to influence textbook illustrators.

The authors of general texts have in common one particularly difficult problem: they must satisfy two quite different goals. Some courses are taught from the text, which must be written so that on page one students enter as novices, become progressively informed as they proceed through the book, and on page 1,000 emerge as biological polymaths. Other courses, however, are centred around lectures, the text being used as supplementary reading and as a reference book. In this case continuity is less important than the encyclopaedic qualities and this means special stress in having the facts up-to-date.

The one first-edition book before me is *Life: The Science of Biology*, by Purves and Orians. While the only way a text can be properly judged is by using it in one's own course, on reading alone this book gets high marks on all the criteria outlined above. It is organized in the conventional way, starting with molecules and ending with populations, and is written in a clear, straightforward manner. Each chapter has a brief but helpful preview and a useful summary at the end which is followed by a short bibliography and a few "study questions". As far as a single mortal could discover, it is up on all the latest, important discoveries. There is perhaps a slightly greater emphasis than usual on evolution and ecology, but the segment covering these topics is excellent. The illustrations are beautiful and the book weighs in at a mere five- and-a-half pounds. It compares favourably with the giants in the field, such as Keeton, and Helena Curtis's *Biology*, 4th Edn (for review see *Nature* 302, 166;

1983), and will no doubt give them stiff competition.

The fifth edition of Kimball's *Biology* is a good, solid and dependable book (what could be better evidence than the fact that this is the fifth edition?). In this instance the author lays stress on the fact that it has been appropriately modernized, and there is every evidence that the new material has been well integrated.

Ritchie and Carola's *Biology*, now in its second edition, is less appealing to me, although I can see that others may find it exactly suited to their tastes. It puts special emphasis on issues relating to human beings and in this sense it is less of a general biology text. For instance, development and reproduction are largely centred around ourselves, and human implications are stressed throughout, from genetics to acid rain. It even includes a discussion of J. F. Kennedy's medical problems and a more alarming one on Napoleon Bonaparte's, with the implication that they explain why he fell asleep during the battle of Waterloo. There are some strange omissions. For instance, there is a clear, although brief discussion of how hormones act on cells, but only peptide hormones are described; no mention is made of how steroids are received by target cells.

I have one major complaint against all these books. For reasons I cannot fully control, I tend to examine carefully what a new text has to say about cellular slime moulds. It was most distressing to discover that Purves and Orians, Keeton and McFadden, and Kimball, all say something that is seriously wrong about these glorious beasts. Ritchie and Carola are the only ones that come away clean by not mentioning them at all.

These errors about slime moulds are part of a wider phenomenon that stems from copying what is said in other texts rather than going to original sources. When I first started to teach general biology years ago, many of the books said the largest plant was a brown alga (a kelp) that reached, by some accounts, a length of 1,500 feet. This bit of misinformation goes back into the last century and was repeated endlessly, in some instances into the 1960s, even though in 1915 T.C. Frye and his co-workers had firmly established the world record was about 150 feet. This is only a slight shift in a decimal point, and eventually the truth will out. Textbooks change mainly by cultural evolution, but in the cases of brown algae and slime moulds there is a closer resemblance to slow genetic evolution.

As an addendum, a word about Pentz's *Biolab Book*. I am no expert on laboratory manuals, but I must say this one is quite special. It is well written (although the print is hard to read) and has most amusing drawings. I sincerely hope the laboratory exercises are equally good and turn out to be useful. □

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● Oxford University Press have re-issued in paperback a biography of one of the twentieth century's most notable and controversial biologists, J.B.S. Haldane. The book, simply entitled *J.B.S.*, is by Ronald Clark and first appeared in 1968. Price in paperback is £3.95.

Paths to physiology

Ole H. Petersen

Human Anatomy and Physiology.

By Eldra Pearl Solomon and P. William Davis.

Saunders: 1983. Pp.794. Hbk \$33.95, £24.95; pbk £11.95.

Animal Physiology: Mechanisms and Adaptations, 2nd Edn.

By Roger Eckert and David Randall.

W.H. Freeman: 1983. Pp.830. Hbk \$32.95, £30.95; pbk £19.95.

Animal Physiology: Adaptation and Environment, 3rd Edn.

By Knut Schmidt-Nielsen.

Cambridge University Press: 1984.

Pp.619. Hbk £15, \$29.95; pbk £12.95.

General and Comparative Physiology, 3rd Edn.

By William S. Hoar.

Prentice-Hall: 1983. Pp.851.

\$32.95, £31.30.

Human Physiology.

Edited by Robert F. Schmidt and

Gerhard Thews.

Springer-Verlag: 1983. Pp.725. DM98, \$39.20.

PFLÜGER, the founder of the oldest extant physiological journal, insisted that as the science of living matter physiology could not be divided into physiological physics and physiological chemistry. However, to a certain extent this has happened. Physiology courses now generally do not include biochemistry, the practice being reflected in most current textbooks. Nonetheless the borders between physiology and biochemistry are ill defined, particularly in areas such as membrane transport, neurobiology and endocrinology — all amongst the growth points in the life sciences.

The authors of the five basic textbooks under review follow different paths in this respect. The most elementary of the books, *Human Anatomy and Physiology*, includes substantial amounts of (basic) biochemistry, whereas at the other end of the spectrum *Human Physiology* contains virtually no biological chemistry leaving, for example, the chapter on endocrinology with bleeding wounds.

Human Anatomy and Physiology is a new textbook (as opposed to a new edition), mainly designed for introductory courses in the health sciences. It has a trendy appearance, with many coloured diagrams and various boxes with special inserts, and at the end of each chapter one finds summary, "post-test" and review questions. Each chapter is also preceded by an outline, learning objectives and key terms. The illustrations are of good quality; many are excellent. The book is comprehensive at a very basic level and could well become a popular choice for nurses as well as different categories of medical technologists. There are, however,

some misleading statements and factual errors. These are particularly conspicuous in the areas of cellular physiology and should be corrected in future editions.

The two versions of *Animal Physiology* as well as *General and Comparative Physiology* are designed for science students taking physiology courses within departments of physiology or zoology. Of the three, Eckert and Randall's book, now appearing in its second edition, is by far the most impressive. It has already acquired a solid reputation which is bound to grow further since the authors do indeed, as claimed in the preface, "develop the major ideas in a simple and direct manner stressing principles and mechanisms. . .". The standard of presentation is very high indeed, the figures being instructive as well as visually attractive. Each chapter ends with a summary and exercises; and in addition to a reading list, directing students to important reviews and books, there is a set of references (on the whole up-to-date) actually cited in the text. Many of the chapters are far superior to the treatment found in other textbooks, including those intended for medical students (for example, Chapter 11 on chemical messengers and regulators contains a clear description of the role of cyclic AMP and Ca^{2+} as intracellular messengers for the action of hormones, something sadly lacking in most other texts). I recommend this book without reservations, first of all to the physiology students it is intended for, but also to medical students as a supplement to their standard reading.

Schmidt-Nielsen's well-known *Animal Physiology*, now in its third edition, is a somewhat briefer account with more emphasis on adaptation and environment. The text is straightforward without any gimmicks. However chapters dealing with several important areas, for example neurophysiology, are lightweight. A particularly unfortunate figure, schematically illustrating synapses that effect electrical and chemical transmission, has the latter labelled as a "tight junction"!

Hoar's *General and Comparative Physiology*, also in its third edition, is comparable in length and cost to Eckert and Randall's *Animal Physiology* but cannot stand up to the competition. The layout, quality of figures, clarity and depth of treatment as well as the references are all inferior. It is nevertheless a solid book, containing good chapters on homeostatic mechanisms and endocrine regulation of reproduction.

Human Physiology is a good English translation of Schmidt and Thews's textbook which has appeared in numerous German editions. Although there is already an impressive number of English and American textbooks for the large medical market, this one could eventually become a best-seller. The presentation is excellent. The figures are of a uniformly high standard (mostly they are schematic, in black, grey, white and red, but with

original records included as required), genuinely help understanding and are well integrated with the text. The chapters on neuroscience and the accounts of the cardiovascular and respiratory systems are in my opinion superior to those in comparable medical physiology textbooks. This will be good enough for some potential purchasers and *Human Physiology* will be seen by many dental and medical students as a good first choice of textbook on the subject.

It is therefore annoying that it is not possible to recommend the book without reservations. The balance is not right. Almost exactly half of the book (328 pages) is taken up by the nervous system. As a consequence, other areas — some of them of immediate importance in the study of internal medicine — are unacceptably neglected. Two areas are very badly hit: the gastrointestinal tract (22 pages) and the endocrine system (29 pages). The chapter on the function of the gastrointestinal canal conveys the completely wrong impression that this field is a backwater of physiology on which new technologies, for example in cellular physiology, have made little impression. The references listed at the end of the chapter do not help very much as they are rather dated. The endocrine chapter, however, is the worst. Incredibly, mechanisms of action receive only about 200 words, with the unsatisfactory excuse that this is an area discussed in biochemical and specialized texts. This certainly is not in line with the course requirements of most medical-school physiology departments in the United States and Britain. The sections on special endocrinology are also too brief and are unsatisfactory as seen, for example, in the superficial treatment of the pancreatic hormones and the control of blood glucose. These deficiencies must be rectified if the book is to achieve the success it otherwise so richly deserves.

Authors of physiology textbooks are not known for taking up new ideas quickly. It is nevertheless sad that although the books reviewed all describe in some detail the electrical membrane characteristics of nerve and muscle cells, proclaiming this area as one in which some of the triumphs in physiology have been won, none of the authors seem to have appreciated the impact that patch-clamp single-channel current recordings could have at the level of undergraduate teaching. It has been my experience that the molecular approach to membrane physiology, with an emphasis on specific ion pathways studied at the level of single-channels, makes it much easier for the student to understand basic electrophysiological concepts. Do physiology textbook authors have to wait until Neher and Sakmann have been awarded the Nobel Prize before they take into account this revolution in neurobiology? □

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Mathematics applied

M. B. Usher

A Biologist's Basic Mathematics.

By David R. Causton.

Edward Arnold: 1983. Pp.216.

Pbk £7.50.

Differential Equations and Mathematical Biology.

By D. S. Jones and B. D. Sleeman.

George Allen & Unwin: 1983. Pp.339.

£15, \$35.

Introduction to Dynamics.

By Ian Percival and Derek Richards.

Cambridge University Press: 1983.

Pp.228. Hbk £18.50, \$35.50;

pbk £7.95, \$14.95.

LOOKING at this collection of books as a biologist, it rapidly becomes apparent that they divide into three steps of mathematical complexity. D. R. Causton is a biologist who has written an introductory text containing material for a first-year biology undergraduate who has an average pre-university education in mathematics. The second step is D. S. Jones and B. D. Sleeman's text: this book is a mathematical introduction to differential equations with biological applications, clearly written by mathematicians with biologists in mind. Finally, I. Percival and D. Richards's book is mathematical, with few concessions to the biologist. Biology features in it purely as one area of the application of stability theory.

Many topics are considered in *A Biologist's Basic Mathematics*: numbers, indices, logarithms, a whole diversity of straight line and curved functions (including the exponential curve and plant growth curves), calculus and matrix algebra. The text is generally clearly written and, section by section, there are worked examples of the application of the mathematical techniques in some area of biology. I would, however, criticize the lack of biology in some of the explanations. For example, first and second derivatives are introduced by considering a marble rolling following acceleration (a) given by $a = 3(T-2t)$, where t is time and T is the position where the marble comes to rest. The reason for this particular acceleration in a non-biological example is not mentioned when the example is introduced. The book contains no statistics, instead concentrating on the models underlying many biological concepts.

The introductory chapter of *Differential Equations and Mathematical Biology* contains examples of three areas of the application of differential calculus — growth of cells in culture, growth of populations and metabolism of drugs. The second chapter, which is particularly well written, then explains much of the terminology. The remaining 12 chapters each address either a topic in differential equation theory (first-order systems,

partial differential equations, evolutionary equations), or an application in biology (heart beat, tumour growth, nerve impulse transmission, epidemic spread, predator-prey interactions). For most biologists, this is a reference book where a selection of four or five chapters (introductory, technical, and in their research area) will suffice.

Although it was with some trepidation that I looked at *Introduction to Dynamics*, the first chapter was reassuring. Here, Percival and Richards give a beautifully clear introduction to dynamics, discussing why scientists should be interested in the stability properties of their equations, and explaining the terminology. Although short, it provides a useful education for many non-numerate biologists. The following two chapters are also introductory, dealing with transformations, including rotations, and second order autonomous systems. Thereafter, the going becomes tougher — Hamiltonian dynamics come to the fore, and the fourth chapter introduces such systems with one degree of freedom. A collection of five chapters then deal with the more

mathematical topics — Lagrangians, the theory of transformations and perturbation, angle-action variables, oscillation — and the two concluding chapters are more applied, covering linear systems and chaos, the latter making reference to the work of R. M. May in the mid-1970s.

Despite their different mathematical levels, all three books follow the common pattern of having a collection of exercises at the end of every chapter, and of making virtually no reference to other published sources (except for Chapter 3 of *Differential Equations*). This latter point makes it more difficult to delve deeper into the subject, and in particular mars Causton's introductory book. The treatment of the exercises also reflects the levels of the book: Causton gives reasonably complete notes and solutions, Jones and Sleeman give rather bare answers, whilst Percival and Richards provide no solutions at all. □

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Nervous comparison

Adam M. Sillito

Test Your Understanding of Neurophysiology.

By R.W. Murray.

Cambridge University Press: 1983.

Pp.291. Hbk £25, \$49.50; pbk £8.95, \$16.95.

Neurobiology.

By Gordon M. Shepherd.

Oxford University Press: 1983. Pp.611.

Hbk \$49.50, £35; pbk £15, \$24.95.

Physiology of the Nervous System.

By David Ottoson.

Macmillan Press, London/Oxford

University Press: 1983. Pp.527.

Hbk £30, \$45; pbk £15, \$27.95.

The Structural Basis of Neurobiology.

Edited by Edward G. Jones.

Macmillan Press, London/Elsevier, New

York: 1983. Pp.417. Pbk £12, \$19.95.

WE ARE rapidly approaching the situation where students may justifiably claim to be overwhelmed by the choice of neuroscience textbooks. This abundance of riches is misleading, however. With some exceptions, even quite standard neuroscience texts have a notably different emphasis; indeed, certain of them are of very limited appeal and can only be considered for specialist courses.

The new book by R.W. Murray falls into the latter category, despite its wide-ranging invitation to *Test Your Understanding of Neurophysiology*. This unusual, and in its early chapters rather archaic text, covers a restricted range of topics derived from the course given in the author's department at

the University of Birmingham, U.K. The approach is somewhat biophysical, but lacks depth and coherence.

The unique feature of this book is its subdivision into an initial set of introductory chapters, followed by a section which poses problems based on specific experimental situations which challenge the application of the knowledge acquired by the student. Whilst all this is very laudable, the result is a rather dry and inconsistent presentation which is unlikely to stimulate the interest of any but the most dedicated of students. On the other hand, the book may be useful to course organizers seeking to utilize some of the tests so thoroughly analysed in its latter part.

In contrast to the limited scope of Murray's book, Gordon M. Shepherd's *Neurobiology* attempts the daunting task of constructing a unified overview of the functional organization and development of vertebrate and invertebrate nervous systems. Anyone who has read Shepherd's *Synaptic Organisation of the Brain* (Oxford University Press, 2nd Edn 1979) will turn to this new work with high expectations; they will not be disappointed. The comparative information, so lucidly developed, provides a perspective which has not been attained by anyone else writing at this level; it certainly goes some way to fulfilling the author's stated objective of encouraging the synthesis of a body of basic principles in neurobiology. This is not a book that loses its instructive impact in a morass of generalities, the material being covered in sufficient depth for it to be more than adequate as a basic text for many undergraduate neuroscience courses. The information on higher vertebrates and man is not only well elaborated and up to date, but is presented in a way

that encourages thought and comparison with other nervous systems. Another attractive aspect of the book is the effort that is made to delineate the link between neuronal organization and behaviour.

There are some gaps, however. For example, the coverage of the visual system seems unreasonably brief given that this is one of the areas in neuroscience where we are closest to establishing a fusion of structure and function. Is this a reaction to the over-representation of the visual system in other texts?

Although Erasmus Darwin, amateur meteorologist, physician and poet, is well known as the author of *Zoonomia*, I did not realize that he was also responsible for the over stimulation theory of pain. Delightful titbits of information such as this abound in David Ottoson's scholarly and very readable *Physiology of the Nervous System*. In approach the book is rather old-fashioned, with a strong historical content, and there is an abundance of clinical information for medical students; but it is thorough and generally up to date with few obvious inaccuracies, and should encourage interest in the subject.

On the debit side, much of the information is organized in a way that necessitates reading a large part of the book to grasp such primary topics as motor control, somaesthesia and pain. I suspect that this will not endear it to weaker souls amongst the hard-pressed medical students seeking to keep aboard the daily treadmill of lectures and tutorials. Nonetheless, for those prepared to work with its format the book has much to offer and is likely to be retained for reference.

Most neuroanatomical texts at the undergraduate level tend to concentrate on the organization of tracts and the gross morphology of the brain at the expense of any detailed treatment of its cellular organization. This is unfortunate; our growing appreciation of the function of the nervous system requires an understanding of the patterns of its synaptic connectivity. This need has been anticipated by the editor of *The Structural Basis of Neurobiology*.

This book is derived from parts of the latest edition of another textbook, *Histology: Cell and Tissue Biology* (see p.121 for review). Two sections from the original work, "The Cell" and "Nervous Tissue", are included together with a new account of the special sense organs. Whilst it does not cover any really new ground, the book provides a thorough background to neurocytology and certainly achieves the editor's objectives. It is a pity that the opportunity was not taken to update the section on nervous tissue in light of some of the issues raised by current functional and neurohistochemical studies; but perhaps the next edition will see to that. □

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Past psychology

P.E. Bryant

A History of Western Psychology.

By David J. Murray.
Prentice-Hall: 1983. Pp.428.
\$35.05, £24.65.

STUDENTS of psychology tend to be fascinated only by the most recent theory or experiment. Everything that went before is rapidly discarded and even despised. There are a number of reasons for this easy contempt for the past. One is that there have been many blind alleys in psychology. But perhaps the most pressing reason for the general unpopularity of courses on the history of psychology is that it is so forbiddingly complex. There cannot have been a subject about which so much has been written from so many points of view.

A mere glance at David Murray's account of psychology's precursors is enough to show that. He starts with pre-Socratic philosophy, gives a fair account of Plato and Aristotle and their immediate successors, has some interesting things to say about the Middle Ages and early Christianity, and then settles comfortably

into his stride with the familiar giants — Locke, Hume, Descartes, Leibniz and Spinoza. Not until half-way through the book does he reach the emergence of empirical psychology at the end of the nineteenth century, and even then he has much to say about psychologists such as James who did no experiments.

The book, then, is really about the history of ideas in psychology. It gives a fair and clear, though often necessarily hurried account of them, and will be a great help to anyone who wants to follow the many different currents of thought that led to the modern conception of the subject.

There are a few weaknesses. The author does not make many connections, though several call out to be explained. For example, what is the point of talking about Kant without describing the great influence that he had on psychologists such as Piaget? And why juxtapose accounts of gestalt psychology and behaviourism without also describing the formidable struggle between these two violently opposed schools of thought — a struggle which is relevant to a number of psychology's current concerns? □

P.E. Bryant is Watts Professor of Experimental Psychology at the University of Oxford.

Ideas of cognition

K. Stenning

Thinking, Problem Solving, Cognition.

By Richard E. Mayer.
W. H. Freeman: 1983. Pp.426.
Hbk \$27.95, £25.95; pbk \$14.50, £16.95.

Learning and Memory.

By Donald A. Norman.
W. H. Freeman: 1982. Pp.129.
Hbk \$16.95, £15.95; pbk \$8.95, £7.95.

The Psychology of Cognition, 2nd Edn.

By Gillian Cohen.
Academic: 1983. Pp.277. Hbk £17, \$32;
pbk £8.50, \$16.

THE problem of choosing a textbook for a course in cognitive psychology is classified, as at least the first two of these books would tell us, as one with ill-defined initial state and ill-defined goal state. However, our three authors' protocols allow us to reconstruct a good deal of their diverse conceptions of what the problem might be.

Mayer conceives of it as a problem of surveying a space — namely, experimental psychological research on the higher mental processes — and therefore requiring an organizing principle, that of history. As a surveyor he is fairly comprehensive but the history is of a peculiarly narrative kind. The twin poles of association and gestalt are used to introduce the topic, with deference to the ancestors' beliefs that they were two mutually exclusive theories of mind. They are, apparently, superseded by a cringe-

worthy construction "meaning theory" which seems later to be somehow distinct from "schema theory" but neither seems well enough defined for students to judge the issue for themselves. Mayer is best where the field is best organized, worst where it leans most heavily on related intellectual endeavours such as logic. It is the fate of the surveyor to reflect the object of survey.

Norman sees the problem as one of reminiscence — in pursuit of research past — and who could blame the reminiscence for belonging mainly to its pursuer? What searcher in memory's vault would be lured towards the comprehensive? Here is a highly personal conception of a subject that might well raise, for the beginner, some of the important questions of the field, and so much the better for omitting a too-hasty attempt to provide the details of the answers. If students were to be required to develop much sensitivity to theoretical alternatives, they would need to be referred elsewhere, outside Norman's own texts perhaps — a favour he immodestly declines them.

Where better to turn than to Gillian Cohen's primer on debate — the thinking students' guide to the study of thinking? She begins with an account of the rules and principles — the organizing issues — and then pursues them through several well-chosen areas of cognitive psychology with a merciless eye for all those psychological theories which pass each other by without the much-heralded clash of armour. She eschews comprehensiveness in favour of including far more — an idea of how the

sundry intellectual approaches fit together. The result is a much harder book that requires and encourages exercise. Here, for example, is an account of what association and gestalt might really have to do with one another. It is also a book that begins to be able to offend, or at least to engender disagreement. It seems to me that the early discussion of the "computer metaphor" fails to benefit from the still earlier discussion of the hardware-software distinction. The relevant comparisons are between subject and software, and so the disanalogies between wetware and hardware do not score their points.

Along related lines, I would complain about the message of the last chapter on the study of the brain's implementations. The chapter reveals two things: what little we do know about the brain comes because we have studied language functionally; and how little the study of the brain has taught us about the functional organization of language behaviour. But then this book puts at least the brighter students in a position to complain for themselves. □

K. Stenning is a Lecturer in the Department of Psychology and in the School of Epistemics at the University of Edinburgh.

Traces of science

David M. Knight

Science and the Making of the Modern World.

By John Marks.

Heinemann Educational: 1983. Pp.507.

Hbk £19.50, \$23; pbk £9.50.

Science and Social Change 1700-1900.

By Colin A. Russell.

Macmillan Press, London/St Martin's

Press, New York: 1983. Pp.307.

Hbk £15, \$25; pbk £5.95.

JOHN Marks's boldness in depicting world science from the ancient Greeks to the present has paid off. His lavishly-illustrated textbook, written in easy, didactic prose and based on secondary sources, describes how disciplines have changed and what is special about science. He picks out the stars: we have Lyell, Müller, Edison and the Royal Society, but not Murchison, Baer, Ohm or the BAAS. A genial tone of common sense pervades the volume, leading us to the conclusions that science is now indispensable, whether we like it or not; that its characteristics of open criticism, pluralism and tentativeness fit best with democratic societies; that government direction will not work; and that what the history of science shows is evolutionary rationalism and spontaneous ordering — that is, trial and error with piecemeal improvement, but no simple and universal method.

This tone might alarm those, to the left of Marks, who see science primarily as a

method of social control, used to achieve or retain power and to generate deference in the working class. Especially for science during the Industrial Revolution, literary and philosophical societies have been seen as legitimizing élites while mechanics institutes produced "Uncle Tom" workmen. Colin Russell engages with this view — which can sometimes seem plausible — in his new book. In contrast to Marks, Russell concentrates on a 200-year period, and on Britain, the first industrial nation, though with chapters on France and Germany for comparison. Because of this narrower range, he can go deeper into questions, and his study is based on original material. It is particularly strong on chemists and naturalists, and their local and national societies.

Russell and Marks agree that early technology was a matter of trial and error rather than of science. Although the benefits of science were therefore hard to see much before 1850, Russell shows that the rhetoric of "useful knowledge" proved very powerful, not only with the mighty but also with Radicals and Dissenters. No distinction was made in the early nineteenth century between pure and applied science — to be applied, science had to be understood, which was (and is) difficult — but "science" and "trade" were distinguished. Elementary courses at mechanics institutes minimized theory, but did not confine themselves to awe-inspiring sciences such as astronomy: disorderly ones, chemistry and engineering for example, were also prominent.

Until recently we took government support for research and for universities for granted, but Russell shows how controversial this was a century ago. The (sometimes wealthy) amateur remained important; government money, as at Kew, meant control; self-help, using string and sealing-wax, was a living tradition; and private benefactions seemed a less sordid source of funds than politicking. The complexity of scientific institutions, and the impossibility of making easy generalizations about them, emerges from Russell as he charts the growth of specialization and of professionalization in its various senses.

For anybody wanting to know how scientists came to be the way they are, as science changed from a hobby to cultural leadership, his book can be unreservedly recommended. For the student, there is something to be said for the enormous canvas and broad strokes of Marks; but there are also good arguments for the close study of a briefer period, where we can see the normal as well as the outstanding and really come to grips with change and continuity through time. Both books well justify the view that to understand the complex activity called science we need an evolutionary, historical, perspective. □

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Practical ends

David Cohen

New Essential Psychology, a series*.

General editor Peter Herriot.

Methuen: 1984. Each book pp.150-200.

Pbk £2.95, \$4.95.

Learning Theory and Behaviour Modification.

By Stephen Walker.

Cognitive Development and Education.

By Johanna Turner.

Selves in Relation: An Introduction to Psychotherapy and Groups.

By Keith Oatley.

WHEN psychology developed into a distinct discipline around 1900, many of its early stars thought that it would blossom into the science to affect life. It was not long, however, before psychologists scurried back into the laboratory. Indeed, in the 1970s one social psychology journal published annual figures which showed that well under 5% of studies examined behaviour in the field. Since then, however, there has been a modest shift in attitudes, and many psychologists have again begun to take an interest in real behaviour in the real world.

The blurb for *New Essential Psychology* blazons the fact that it is out "to demonstrate the futility of [the] distinction" between pure and applied research. This sounds good. The next part of the blurb is, however, just untrue. We are told that "most major advances in psychology have resulted from its use for practical purposes". In fact, most work in learning theory, one of the most fruitful areas in psychology, has theoretical roots. And while one currently exciting field, developmental psychology, suggests that babies are born much more able than we have thought, there was no practical purposes to inspire the work. Much of it was a reaction to Piaget's ideas and yet another round in a long theoretical battle.

Having become suspicious about the philosophy of the series, I was pleasantly surprised by Stephen Walker's *Learning Theory and Behaviour Modification*. Walker gives a good account of the latest research, including that on the limits of animal intelligence. There are also entertaining chapters on how Watson affected advertising, on Skinner's behaviourist Utopia and on the way that learning theory did lead to behaviour therapy which may well be the best cure for phobias. But even Walker fails to comment on the fact that it was work with the rats in the laboratory — all of it stemming back to Pavlov — which led to practical help in the clinic.

The other two books covered here are

*Also available are *Social Interaction and Its Management*, by Judy Gahagan, and *Instinct, Environment and Behaviour*, by S.E.G. Lea.

severe disappointments in their different ways. Johanna Turner's *Cognitive Development and Education* is largely a rehash of the work of Piaget and criticisms of him; Turner does not even cover Piaget's own lamentations about the narrow role of teachers whose cognitive development is often stunted but prefers to take us, yet again, through his ideas. She does tack on a few sections on practical topics such as "school readiness" but this is very traditional book which challenges no old distinctions.

Keith Oatley's *Selves in Relation*, which deals with psychotherapy, is a more novel failure. Much of the book centres around fictional case-histories, so that we now have a distinction between pure, applied and utterly invented psychology. As a fiction writer, Oatley is not very good as his suffering characters are more wooden than the virgins of Barbara Cartland. He is also too concerned with showing off as a radical. One would never guess that psychiatrists carry out much psychotherapy and that most "clients" are seen, in Britain

at least, in the context of some psychiatric care. Oatley tells us as well that he became bad-tempered while finishing the book so that we remember he is a human being whose subjective voice is crucial. We get, too, the now mandatory chapter on Lacan. Given the philosophy of the series, it would have been nice to see some attention paid to why psychotherapy became so popular in the 1970s and to the whole question of why people feel impelled to change themselves. Oatley does offer a sensible account of the research into the effectiveness of therapy for individuals and groups. Ironically, the book is best when it is conventional.

As a whole, the series is a nice "selling idea", as Watson coined the phrase, but it needs rather more thought and imagination in its execution. A challenge to the dichotomy between pure and applied work is a worthy aim; however it will require re-doing much of psychology, not merely re-writing the results with an eye on the real world. □

David Cohen is the Editor of *Psychology News*.

Old Americans

Warwick Bray

Ancient North Americans.

Edited by Jesse D. Jennings.
W.H. Freeman: 1983. Pp.642.
\$27.95, £23.95.

Ancient South Americans.

Edited by Jesse D. Jennings.
W.H. Freeman: 1983. Pp.414.
\$24.95, £21.50.

THE precursor of these two volumes was a single book, *Ancient Native Americans*, published as recently as 1978. In its day this was one of the best textbooks on the market, though some of us (mainly those working in Latin America) grumbled that it was aimed too specifically at North American university students, and also complained that the less fashionable areas of South America were ignored.

The pace of archaeological research is so fast nowadays that some revision would, in any case, have been necessary, and the editor has taken the opportunity to split the work into two volumes and to commission completely new chapters on the north-eastern United States, the northern Andes (i.e. Colombia and Ecuador), and the south Andes (the Titicaca Basin and adjacent parts of Chile). In addition, Frank Hole contributes a new chapter on "Changing Directions of Archeological Thought", which appears in both volumes; this is one of the sanest and best balanced statements I have read for a long time.

The original chapters, all of them by acknowledged specialists, have been extensively revised in order to incorporate new information, much of it unpublished, so that the reader feels he is getting his news

hot from the field. Several papers go beyond mere textbook summarizing, and are serious contributions to scholarship. Sadly, though, there is still nothing on the Isthmian cultures or the southern part of South America.

As well as Hole's essay, the articles on Mesoamerica and on transoceanic contacts appear in both volumes. So out of the eight chapters in *Ancient South Americans*, three are duplicates. This verges on sharp practice, for the two volumes were clearly designed as a set, and all librarians (if not all students) will want to buy both of them.

In other respects the new version preserves the virtues of the 1978 edition, with up-to-date information, literate text and generous and intelligently chosen illustrations; excellent bibliographies appear at the end of each section. A major theme of these books, and one which will appeal to geographers and ecologists, is the relationship between man and the changing prehistoric landscape. How many archaeology books bother to show the reader what a jiquima tuber or an extinct ground sloth actually look like? Within this general framework, some authors concentrate on problems of interpretation, while others show more interest in lists of artefacts, dates, sites and cultures. Such facts are essential, of course, but they do not speak for themselves, and the sceptic might need to be shown why it is worth the effort of mastering all the detail. This, however, is a minor complaint. At a time when archaeology is becoming more popular, all teachers, university students, and the more informed amateurs, should be grateful for textbooks they can trust. □

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The magnetic past

K.M. Creer

Palaeomagnetism: Principles and Applications in Geology, Geophysics and Archaeology.

By D.H. Tarling.

Chapman & Hall: 1983. Pp.379.

Hbk £25, \$55; pbk £12.95, \$29.95.

PALAEOMAGNETISM is a discipline that has drawn in research workers with both physical and geological backgrounds. In consequence, the subject now covers a very wide range of methods such that no single person can become a master of all of them.

D.H. Tarling, the author of this book, is himself a geologist with many years of research and teaching experience in palaeomagnetism, so one is not surprised to find his chapter on geological applications to be written with authority and the topics to have been well selected. One of the strengths of his book, however, results from Tarling's good fortune to be based in a Geophysics Department which constitutes part of a School of Physics, and he has clearly taken full advantage of the proximity of his physics-orientated colleagues to test out his chapters dealing with the physical basis of the subject, magnetization processes in minerals and rock assemblages, instrumentation and statistical methods. The Department of Geophysics and Planetary Physics at the University of Newcastle has played a leading role in both the development and the application of techniques in palaeomagnetism and rock magnetism through the past quarter century or more, so one could hardly find a better environment from which an authoritative text could be created.

Although Tarling has followed the overall plan of his earlier work *Principles and Applications of Palaeomagnetism*, published by Chapman and Hall in 1971, this is very much a new book. While the chapter titles are almost identical with those of the earlier book, the contents have been considerably expanded and updated, and there is an additional chapter on "Archaeological Applications". Well over 1,000 papers are referenced and a quick browse suggested to me that the book could be considered as a primary text and reference source for the research worker specializing in palaeomagnetism. Further perusal confirmed my initial impression and I recommend it strongly both to the research worker and to the post-graduate student interested in palaeomagnetism. Tarling's new book has no obvious weaknesses and compares most favourably with the current best-selling texts on the subject. □

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The two sides of mineralogy

R.A. Howie

Mineralogy: Concepts, Descriptions, Determinations, 2nd Edn.

By L.G. Berry, Brian Mason and R.V. Dietrich
W.H. Freeman: 1983. Pp.561.
 \$35.95, £30.95.

THE first edition of *Mineralogy* appeared in 1959, so the book has long been due for an overhaul. This new edition has undergone extensive revision, and is intended not only as a textbook for a first course in mineralogy but also as a reference work for geologists, pedologists, solid-state physicists and others who use crystallography or mineralogy in their work.

Additions include brief accounts of the phase rule, phase equilibrium diagrams, Pauling's rules, optical mineralogy techniques, and applications of the electron microprobe; sections dealing with the gnomonic projection and blowpipe analysis have been discarded. Specific locality and production data, previously included in the mineral descriptions, are also omitted.

The text falls into two main parts, the first dealing with crystallography, crystal chemistry, physical and optical properties and determinative techniques, while the second provides descriptions of some 200

minerals. The latter section includes fairly extensive coverage of sulphides and sulphosalts, nitrates, borates, chromates, molybdates, arsenates, vanadates and so on, in addition to the rock-forming minerals of the oxide, sulphate, carbonate and silicate groups. The book may thus be compared with the second edition of M.H. Battey's *Mineralogy for Students* (Longman, 1981; for review see *Nature* 295, 464; 1982) and will be of particular relevance to students of mining and economic geology.

Fairly minor criticisms are that the notes on the optical indicatrix and the determination of optic sign are surely so brief as to provide little help to the student seeking to reproduce some of the optical data in the determinative tables (which form Part III of the book). The concept of extinction angle is mentioned in Part I but no extinction angles are listed in the descriptive sections, though cell parameters and space groups are given for each species.

My overall impression of this second edition is that it has been considerably improved (the diagrams and line drawings are particularly clear) but that it is trying to cover too much. In view of the reluctance of some students to buy more than one book for each course it is no doubt sensible and certainly convenient to include techniques and properties together in one volume, but it leads to a rather superficial coverage of many topics. □

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Ores in context

C.D. Curtis

Geochemistry of Sedimentary Ore Deposits. By J. Barry Maynard.

Springer-Verlag: 1983. Pp.305. DM69,
 \$27.40.

RELATIVELY recently it has been recognized that many economically important metalliferous ore deposits owe their origin to sedimentary processes and, in particular, to chemical processes. These are the subjects of Barry Maynard's new text. The book differs from most others covering economic geology in that orebodies are treated within the context of "normal" rocks and processes rather than in isolation, as geological "rarities".

Six chapters constitute the main body of the book, each dealing with an individual metal or group of metals (iron, copper and silver, aluminium and nickel, manganese, uranium, lead and zinc). A final chapter deals with volcanic-sedimentary ores, which are treated as either divergent or convergent plate-boundary associations. Each but the last chapter follows a similar pattern — an introduction may identify fundamentally different kinds of deposit,

in which case each is treated separately, before Maynard leads on to discussion of mineralogy then geochemistry. Calculated equilibrium phase diagrams are used extensively — some familiarity with these is assumed — and there is also frequent reference to stable isotope geochemistry. Each section closes with descriptions of specific orebodies and an account of how they might have formed.

Brief descriptions of numerous orebodies from around the world are included in the book and given a separate index — separate that is from author and subject indexes. The bibliography is extensive and up to date. I found these features very useful. My initial overall impression was that too much reliance had been placed on equilibrium relationships amongst minerals, but this, perhaps, was unfair since Maynard gives an explicit appraisal of his approach and its limitations in the introductory chapter.

I liked this book: it brings together traditional economic geology and modern geochemistry in a constructive way. I'm sure it will make a good textbook for a graduate course, which indeed is how it originated. □

Charles Curtis is Professor of Geochemistry at the University of Sheffield.

Sediments, processes and time

J.R.L. Allen

Practical Sedimentology.

By D.W. Lewis.
Hutchinson Ross: 1984. Pp.229.
 Pbk \$22.95, £22.25.

Depositional Systems: A Genetic Approach to Sedimentary Geology.

By Richard A. Davis, Jr.
Prentice-Hall: 1983. Pp.669.
 \$48.55, £34.15.

Dynamic Stratigraphy: An Introduction to Sedimentation and Stratigraphy, 2nd Edn.

By Robley K. Matthews.
Prentice-Hall: 1983. Pp.489.
 \$44.50, £31.30.

THE extraordinary growth of interest in sedimentology over the past twenty years continues to provoke the frequent publication of new student texts. Their diversity of scope, aims and methods is a healthy sign in such a young subject, which has still to define its true identity and aims. Is sedimentology about the history of sedimentary deposits (the "stratigraphical" root of the subject), or is it largely concerned with the mechanisms and processes which at every level shape such deposits (the "geophysical-geochemical-geobiological" root)? The three books reviewed here draw strength in very different degrees from these two sources.

In *Practical Sedimentology* we learn that the role of the sedimentologist is "to interpret the history of sedimentary deposits". Dr Lewis's book exemplifying this theme is in twelve chapters, covering environments and processes of sedimentation, textures and composition of detrital sediments, sedimentary carbonates, evaporite deposits, iron in sedimentation, introduction to well-logging, map varieties and suggestions for fieldwork.

It is good to see attention given in a student text to down-hole methods, but my unease about this book stems from its unimaginativeness and narrowness of outlook, and a feeling that the author had not decided whether he was writing a practical manual or a text to cover principles. The range of practical techniques described is limited and their treatment commonly superficial and lacking in illustrations. The lay-out adopted by the publishers is thoroughly confusing. There is no hint that, using simple equipment and readily available materials, the student can explore experimentally many important sedimentary phenomena, and thereby strengthen insight into what is essentially dynamic. *Practical Sedimentology* cannot be said to fit the bill for a modern and all-round manual of do-it-yourself laboratory and field sedimentology.

Depositional Systems is a systematic but essentially conventional treatment of sedimentary environments and facies in the context of stratigraphy. Dr Davis's book is comprised of sixteen chapters, partitioned into four main sections which cover principles, and then terrestrial, transitional and marine environments. The concept underlying the author's treatment is the Brown-Fisher-Scott depositional system, that is, an assemblage of process-related sedimentary facies. This concept, introduced in the 1960s, is widely used in applied sedimentology, and therefore deserves to be put before undergraduates, but perhaps in a more critical manner than we find here. Under this concept, each system or environment is treated systematically, beginning with an account of its modern expressions in terms of processes and sediments, and concluding with an outline of case-histories drawn from the rock-record in various parts of the world.

Depositional Systems is detailed, accurate, attractively written and presented, but essentially descriptive. It is aimed at no particular national market and includes a well-balanced selection of sedimentological examples. My most serious reservation concerns the author's failure adequately to knit his depositional systems together at the basinal level and in the context of modern geodynamical theory; but provided its limitations are recognized,

Dr Davis's book deserves to find a wide audience.

Dynamic Stratigraphy is the second and greatly improved edition of a book which appeared in 1974, and which was the first to include the adjective "dynamic" in its title. The main additions are a lengthy section concerned with the geodynamical context of sedimentation, and the enlargement of another to embrace the evolution of sedimentary environments throughout the entire Phanerozoic. The book is in nineteen chapters, grouped into five parts: an introduction; sediments, time and the stratigraphic record; the geodynamical context of sediment accumulation; dynamics in the context of environment; and dynamics in the context of time.

This attractive book is systematic, exact, quantitative and, above all, concerned with general models and root causes. The author has many perceptive things to say about sedimentology and stratigraphy and their practitioners which deserve to be widely read, discussed and acted on, and not just by university and college students. My only regret is that its intellectual appeal is undermined by an over-emphasis on North American work in the field of sedimentology. □

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Talk about the oil business

B.W. Sellwood

Petroleum Geology.

By R.E. Chapman.

Elsevier Science: 1983. Pp.415. Dfl.115, \$44.25.

PETROLEUM geology is that branch of the earth sciences concerned with the discovery, evaluation and production of hydrocarbons. There is need for a textbook on the subject for senior undergraduates and the more general professional. This book is not it.

As a teacher I require an up-to-date, well-organized account of exploration rationale and methods, prospect evaluation techniques and production procedures, accompanied by example case-histories ranging across the globe. *Petroleum Geology* falls far short of fitting the bill. It is repetitive, packed with homilies, and the organization is poor with general and technical chapters being littered throughout. There are also many inaccuracies; to give just two examples, land plants are asserted to have arisen in the Mesozoic (p.79) and "regression" is stated as being defined as lowering of sea-level (p.9). Some chapters seem strangely dated, relying heavily on older literature (in

Chapter 8, on petroleum reservoirs, only 9 out of the 27 references cited post-date 1970). In places, where things begin to get interesting, the reader is referred to an unspecified "specialist well-log analyst" (p.115).

I cannot detail here my more specific reservations, but a few examples illustrate the range of problems. Sedimentological aspects of well-log evaluation and reservoir plumbing have been largely ignored, the dipmeter being dismissed in 32 lines. The thin account of drilling dwells on outmoded methods (cable-tool drilling) but ignores bit types and turbo-drilling, and dismisses cuttings as being "almost useless" (p.100) — which they are not. Chapter 7, which falls between chapters on borehole logging and reservoir evaluation, rambles through world and state oil-field rankings, the author spuriously applying Zipf's so-called "law" (on salary distributions!). And while some of the technical chapters are not without merit, the material is more fully covered in Schlumberger and Dresser logging manuals.

Perhaps not surprisingly, the intended readership is never identified. Certainly an undergraduate would find this book heavy going at best and misleading at worst, while professional petroleum geologists will already be familiar with what it contains. □

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Rock collection

Reg Bradshaw

Dictionary of Petrology.

By S.I. Tomkeieff.

Edited by E.K. Walton, B.A.O. Randall, M.H. Battey and O. Tomkeieff.

Wiley: 1983. Pp.680. £49.50, \$97.50.

Igneous Rocks.

By Daniel S. Barker.

Prentice-Hall: 1983. Pp.417.

\$49.90, £35.10.

FOLLOWING an abortive collaboration with A. Holmes to produce a second edition of *Nomenclature of Petrology* (1920), S.I. Tomkeieff conceived the idea of a dictionary which would give the etymology of petrological terms, the development of any meanings with time, and references to their first and subsequent use. Unfortunately he died in 1968 before the work was completed, but his widow enlisted the help of three other editors in preparing his notes for the press. It is clear from the foreword and preface to *Dictionary of Petrology* that it is not the work that was originally intended — it is shorter, less complete and less concerned with etymology.

The dictionary is in two parts. The first, larger part, is an alphabetical list of some 8,000 terms related principally to igneous, sedimentary and metamorphic rocks but with some from soils, economic and structural geology included (meteorites are excluded). The second consists of synoptic tables in which terms from the first part are re-grouped into lists covering different subject areas within the wide field of petrology.

Petrological nomenclature, particularly that of igneous rocks, is complex and unsystematic, and some rock names still in use can be traced back to Pliny and Theophrastus though their meanings may have changed. So while any attempt to explain terminology is to be welcomed, it has to be said that the treatment here is uneven at all levels. The derivation of most names of igneous rocks is given, often with a reference to their author and first use, but terms of long standing may be referenced to works of the 1970s while others have neither etymology nor reference. Rocks such as granite and greywacke justifiably receive considerable attention, but others are mere local rocks with local names from such places as Reunion, Java or Chile. Some terms such as pingo, overlap, hiatus and yardang can hardly be considered to be petrological.

Geologist's guide

Field Geology in the British Isles, according to the publisher the first book to cover geological excursions for the whole of the British Isles, has recently been published by Pergamon Press. The author is J. G. C. Anderson, who after a general introduction describes 194 geological itineraries based on a number of centres. Price in flexicover is £8.95, \$16.

Nonetheless this is an interesting book which could, as its editors claim, form the basis for a more comprehensive work. Many could learn and profit from it. For example, it is useful to know that because of its Greek derivation the glassy basic igneous rock should be spelt *tachylite* not *tachylite*, and entertaining to be informed that the god Hermes is remembered petrologically by a rock of undetermined composition. The book is a pleasure to browse through, but I must confess to a slight disappointment that the long-awaited Tomkeieff *Dictionary* is not a more definitive work than it has turned out to be.

Daniel Barker's *Igneous Rocks* was written for geology students who have had basic courses in chemistry and mineralogy. The book covers a wide range of topics, including phase relations, descriptions and occurrences of the main rock groups, the generation of magmas and the relation of magmas to tectonism. It is well written with good diagrams, though some of the photographs are not too clear, and the reference list of some 600 items is to work which is mainly post-1970. The treatment is extensive rather than intensive, but the book gives a good review of the current state of igneous petrology which will be suitable for second-year undergraduates in Britain and for geology majors in the United States. □

Reg Bradshaw is Senior Lecturer in the Department of Geology, University of Bristol.

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Organic assembly

John Mann

Organic Synthesis: The Disconnection Approach.

By Stuart Warren.

Wiley: 1983. Pp.391. Hbk £19, \$38; pbk £7.95, \$15.95.

Workbook pp.550, £6.95, \$13.95.

Advanced Organic Chemistry, Part B Reactions and Synthesis, 2nd Edn.

By Francis A. Carey and Richard J. Sundberg.

Plenum: 1983. Pp.650. Hbk \$59.50, £45.80; pbk \$16.95, £13.05.

Alicyclic Chemistry, 2nd Edn.

By F.J. McQuillin and M.S. Baird.

Cambridge University Press: 1983. Pp.225. Hbk £20, \$39.50; pbk £7.95, \$16.95.

THE publishing fraternity is deeply involved in a love affair with organic synthesis, and these three books are but a few of the progeny of the liaison. It seems reasonable to enquire why so many eminent chemists have suddenly chosen to write about their *grand passion*. Financial inducements apart, I suspect it may be because the art of organic synthesis has reached a critical stage in its development. Many chemists wish to take stock, to scan the horizon for new and yet more challenging targets for synthesis, and to contemplate how these may be assembled in ever more elegant ways.

Stuart Warren's book encompasses most of organic synthesis, but from an analytical viewpoint; it represents the first successful attempt to place E.J. Corey's ideas on "retrosynthetic analysis" before a general chemical readership. The basic strategy is to disconnect the target molecule at selected points in order to recognize potential synthetic intermediates. The best disconnections are those that are formally the reverse of synthetic operations, hence the term "retrosynthesis"; but any chemical bond may be broken to provide a novel structure. Often new chemistry must be devised if the actual synthesis is to proceed via such intermediates, and many of the innovations of the past ten years arose out of efforts to execute some of the obscure transformations suggested by retrosynthetic analysis.

Warren has produced an excellent systematic account of the subject. He includes numerous examples, mostly taken from the medicinal, pesticide and perfumery chemistries, thus providing a sense of purpose for the analyses. The information is given in concise packages interspersed with clear illustrations, and the book is in consequence easy to read and comprehend. This text should become essential reading for all chemistry undergraduates, and is excellent value for money. The accompanying workbook has a complementary set of examples, and is

also very reasonably priced for its size.

Many of the recent developments in the methodology of organic synthesis are included in Carey and Sundberg's book. (Part A, which has already appeared in a second edition, covers most areas of organic chemistry apart from synthesis.) During the 1970s there was an explosive growth in this kind of chemistry, and their first edition (1977) came too early to capture but a part of the action. This has now been remedied by substantial coverage of, *inter alia*, the applications of sulphur, selenium and silicon reagents, an expanded treatment of cycloaddition reactions, asymmetric syntheses, and alkylations, and a new chapter on the interconversions of functional groups via nucleophilic displacements. There are literally hundreds of illustrative syntheses, and problems on synthesis, many drawn from the recent research literature, and references are provided for both types.

This is the most comprehensive and up-to-date book on organic synthesis for the advanced undergraduate, and will, I am sure be widely read. However, its sheer weight will preclude casual reading on the train or in the bathtub. There are several other books — such as W. Carruthers's *Modern Methods in Organic Synthesis* (Cambridge University Press, 1978) and R.K. Mackie and D.M. Smith's *Guidebook to Organic Synthesis* (Longman, 1982; for review see *Nature* 302; 189; 1983) — which provide excellent and more lightweight (both in mass and content) alternatives.

In chemical analysis, most targets can be considered as an assembly of rings (and associated functionality), appendages and stereochemistry; in synthesis, formation of the rings is usually the critical part — the right choice of method can establish correct functionality and stereochemistry, as well as the foundations for subsequent appendage application. A thorough understanding of alicyclic chemistry is a prerequisite for those who wish to prepare rings, and the book by McQuillin and Baird seeks to satisfy this need.

Once again there have been numerous advances since the first edition of the book appeared in 1972 — not least, almost the whole of prostaglandin chemistry with its attendant demands on cyclopentane chemistry. This new edition thus contains extra examples of the applications of modern alicyclic chemistry in the synthesis of natural products, though some of these examples are not as relevant as they could have been. Special treatment is also given to Baldwin's rules for cyclization and to the frontier orbital treatment of Diels-Alder reactions, but the classical work on conformational analysis and associated topics is still pre-eminent as it should be. Overall the book provides a useful, if somewhat unexciting, introduction to alicyclic chemistry. □

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Under analysis

D. Thorburn Burns

Quantitative Chemical Analysis.

By Daniel C. Harris.

W.H. Freeman: 1982. Pp.748.

\$31.95, £27.50.

TEACHERS of analytical chemistry will find this book to be useful and scholarly acquisition for their personal libraries. Whether or not they will recommend it for their courses is less certain as this depends, as for any teaching text, on how closely it fits their views of the subject and their syllabus. Interestingly, the text has already been evaluated by use, as it was developed from a manuscript used for two years at Franklin and Marshall College, mainly by life science majors but with some chemists.

Only a limited number of topics are included: statistics, chemical equilibrium, acid-base chemistry, electrochemical equilibrium, electroanalytical methods spectrophotometry, methods of separation and a set of 18 experiments. The text is beautifully laid out with wide margins, allowing the author to insert extra diagrams, data, side comments, additional explanations etc. without breaking the flow of the main text. Numerous worked (and fully explained) examples and boxes to deal with subsidiary topics (such as logic

of approximations, meaning of negative pH, descriptions of demonstrations) divide the text into manageable sections.

The discussion of errors and the introduction to statistics are very clear, but the F and (Chi)² tests should have been included. The clarity and the depth of treatment of solution equilibria and the various applications are particularly commendable. The treatment of electroanalytical chemistry is likewise excellent and includes some very up-to-date items such as photo-assisted electrolysis and optically transparent thin layer electrodes.

By contrast, the sections on spectrophotometry and atomic spectroscopy are less detailed and hence less authoritative, although both contain some useful teaching material. Basic chromatography theory is developed clearly and fundamentally via the Craig countercurrent distribution process, although the discussion of specific forces of interaction in both liquid and gas chromatography is not detailed enough to allow development of separations *ab initio*. Some very interesting experiments are described, and each chapter is supported by a collection of exercises, problems and references to further reading. Overall, within its limits, this text is a refreshing addition to the literature. □

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Questions of insight

S.F.A. Kettle

Problems in Molecular Structure.

Edited by G.J. Bullen and D.J.

Greenslade.

Pion: 1983. Pp.466. £16, \$32.

IN *Problems in Molecular Structure* G.F. Bullen and D.J. Greenslade have compiled an excellent book, one which post-graduates in particular but also senior undergraduates on specialist courses will find to be most useful. An especially attractive feature is that each of the problems considered is accompanied by a very detailed answer; thus the average space devoted to a problem and its solution is in excess of two pages.

In all, 25 subject areas are included with an average of about eight questions and answers on each, the range being from three questions on solid-state nuclear magnetic resonance and on mass spectrometry through to 14 on ultraviolet and visible spectroscopy. The subject areas are further broken down into seven topics — symmetry, diffraction (including electron diffraction by crystals), vibration-rotation spectroscopy, electronic properties (ranging from photoelectron spectroscopy through ORD/CD to electron spin resonance and magnetic and electric multi-

polar moments), nuclear spectroscopy, mass spectrometry, and structure and energy. The final one of these contains what is possibly the last contribution by the late C.A. Coulson, who has written a section on wave functions and bonding.

In about one-third of the sections there is a brief overview of the subject area which precedes the problems themselves. I found these introductions helpful and would have welcomed more of them, though they may be of less importance to the student seeking problems in a subject area which is currently being studied. In a book such as this it seems inevitable that space will be devoted to the problem of units. And, indeed, in addition to an appendix this is a topic covered in three of the overviews. Both here, and in the notations adopted in different sections, the editors have taken a broad-minded view which has resulted in a number of inconsistencies; these, however, are unlikely to represent a problem of their own.

The book will probably be read in a piecemeal fashion and is written with this in mind — answers tend to be free-standing, with little cross-referencing between sections. Overall, my impression is a very favourable one — even experts in a particular field will gain in insight by working through the relevant section of the book. □

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Physical attributes

John Avery

Basic Physical Chemistry.

By Walter J. Moore.

Prentice-Hall: 1983. Pp.711. Hbk \$45.85,

£32.25; pbk \$25.95, £12.95.

Physical Chemistry, 2nd Edn.

By Ira N. Levine.

McGraw-Hill: 1983. Pp.890. \$54.95,

£27.75.

Physical Chemistry, 3rd Edn.

By Gilbert W. Castellan.

Addison-Wesley: 1983. Pp.943. Pbk

\$33.95, £14.95.

G.N. LEWIS once defined the scope of physical chemistry as including "everything that's interesting". Although that may have been an exaggeration, the range of topics covered in these three undergraduate physical chemistry texts is impressively wide. Besides chemical thermodynamics, electrochemistry, statistical mechanics, introductory quantum chemistry and crystallography, we have specialized topics such as biological membranes, bioelectrochemistry, enzyme catalysis, photoelectron spectroscopy, molecular spectroscopy, electron spin resonance, nuclear magnetic resonance, group theory, photochemistry, solid state theory and theory of the liquid state — even nerve conduction (Moore), photosynthesis (Castellan) and nuclear reactions (Levine).

All of this material ought to be part of the general background knowledge of serious students of the physical or biological sciences, but one can sympathize with those of them daunted by such a profusion of topics. In mitigation each of the three authors writes with sympathetic understanding of the difficulties likely to be encountered by undergraduates. They have included hints on methods of study, and two of them (Castellan and Levine) give lists of answers to the exercises. Levine also emphasizes important concepts by the use of bold type, includes a summary at the end of each chapter and leaves space for marginal notes.

All of the books are new versions of respected and widely-used textbooks, Moore's being a shortened and re-written version of his earlier *Physical Chemistry*. Many of the figures from that book reappear here, as does some of the text; but so much has been revised that it is correct to speak of a new book rather than a new edition. Moore's standpoint is closer to chemistry than to physics, and his book contains many tables of chemical properties. His method of exposition is descriptive rather than mathematical, and he often states results without giving theoretical derivations. This makes his book the most elementary of the three. By contrast, Levine, and to an even larger extent, Castellan, formulate the material in a mathematical way, so that the physics

comes out clearly; and they give rigorous theoretical derivations wherever possible. Both Castellan and Levine include helpful reviews of the necessary mathematics.

In the new edition of Levine, the two chapters dealing with solutions have been rewritten in such a way as to reduce the number of equations. Both Levine and Castellan have added many worked problems; indeed, the 750 problems in Castellan's third edition roughly doubles the number included in its predecessor. The new edition of Levine also contains sections treating a variety of recently-developed experimental techniques, such as ion cyclotron resonance, field-ion microscopy and photoacoustic-spectroscopy.

Any author of a physical chemistry textbook is faced with a dilemma in organizing his material. The main body of the course must be devoted to chemical thermodynamics. If sections on the kinetic theory of gases and statistical mechanics are placed early in the book, the student is helped in his understanding of thermodynamics. However, one cannot develop statistical mechanics without first saying something about quantum theory; and quantum theory is a difficult topic which

most authors would prefer to put towards the end of their books. These three authors respond differently to the problem. Levine discusses statistical mechanics in one of his later chapters, after the sections on quantum theory. Castellan places the classical derivation of the Maxwell-Boltzmann velocity distribution early in his book, while leaving the part of statistical mechanics which depends on quantum theory until one of his last chapters; and Moore includes a section on quantum theory very early in his book.

All three of these excellent and clearly written textbooks deserve to be considered by anyone planning a course in physical chemistry at the undergraduate level. They not only guide the student safely through the fundamentals, but also introduce him to exciting areas of current research. Personally, I prefer the more exact and mathematical style of Castellan and Levine to the descriptive treatment given by Moore, but the choice of book will depend on the level of the course being planned. □

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The inorganic core

Jon McCleverty

Concepts and Models of Inorganic Chemistry, 2nd Edn.

By Bodie Douglas, Darl H. McDaniel and John J. Alexander.

Wiley: 1983. Pp.800. Hbk £34.15, \$47.85; pbk £10, \$16.

Inorganic Chemistry: A Unified

Approach. By William Porterfield. Addison-Wesley: 1984. Pp.688. \$33.95, £14.95.

BECAUSE of the wide range of inorganic chemistry, and constraints on time, one must be selective in the aspects of the subject presented to undergraduates. So it is reassuring that, on both sides of the Atlantic, there is a common perception of the core of "inorganic" knowledge that every graduating student of chemistry should know. For example, both of the books reviewed here regard as essential a discussion of atomic structure and periodicity, bonding and some aspects of thermodynamics and mechanism, including photochemical processes. Coordination and organometallic chemistry have a definite place and may be linked to homogeneous catalysis. There is also a recognition that limited amounts of descriptive chemistry are necessary, and that attention must be paid to developments in cluster, solid-state and bioinorganic chemistry.

In Douglas *et al.*'s *Concepts and Models of Inorganic Chemistry* the emphasis is on theories and rationalizations rather than on a systematic discussion of the elements,

and so the reader will not find much comment on the synthesis of inorganic compounds or indeed on many of the unsolved mysteries of this challenging branch of the subject. Of particular note, however, is the excellent résumé of symmetry and Group Theory, and the section on solid-state chemistry is very well-illustrated, going satisfyingly beyond most other textbooks pitched at the same level. Notwithstanding the remarkable lack of any reference to magnetism, coordination chemistry is effectively covered, but the section on organometallic chemistry is disappointingly short, with omission of certain important aspects of main-group organometallics. The diagrams and tables are clear and easily readable — possibly because of the book's unusual shape (195 x 240 mm) — but the index is a little confusing, there being reference to compounds which appear only in the (useful) problem sections and not in the text.

In *Inorganic Chemistry*, Porterfield observes that some of the most exciting developments in the field have come from the movement towards integration of previously distinct areas, for example polyhedral boranes and transition metal clusters. Because of this, he suggests that a new organizational principle may be necessary in the teaching of the subject. His book, which is produced to a slightly higher quality than the Wiley text, is in five parts, the first of which is largely introductory. The next two are concerned with the "statics" and "dynamics" of main-group chemistry, and Parts Four and Five with similar aspects of transition-metal chemistry. The reactions of main-group compounds are presented with a strong

thermodynamic flavour, and include significant discussion of redox activity and non-aqueous solvent behaviour. Magnetism is described, and organometallic chemistry and catalysis are thoroughly discussed. One of the most exciting features of this book is the chapter describing photochemical reactions of transition metals.

The main differences between the two books relate more to presentation than to philosophy. Indeed, the authors of *Concepts and Models* actually come closer in some areas to reaching the goal of integration than does Porterfield, especially when dealing with aspects of inorganic solids and cluster chemistry. While covering much of the same material as Douglas *et al.*, Porterfield assembles it differently and in places fails to take full advantage of the unified approach he has espoused. Thus the synthesis of material within the confines of main-group or transition-element chemistry is good, often imaginative, but true unification should dissolve this artificial and partly historical division. By far the clearest difference between the books relates to Group Theory. Porterfield has made the explicit decision not to include accounts of its use. In my view this is most unfortunate — much of contemporary inorganic chemistry relies on the rationalization and handling of spectroscopic and bonding information via Group Theoretical tools.

Both of these books include some more advanced material than Huheey's most readable *Inorganic Chemistry* (Harper & Row, 3rd Edn 1983) — which, incidentally, also omits Group Theory. They are not as heavy going or as selective as Purcell and Kotz's molecular-orbital orientated book, *Inorganic Chemistry* (Holt-Saunders, 1977), but offer far more helpful teaching and explanatory material than *Advanced Inorganic Chemistry*, the comprehensive research-orientated work of Cotton and Wilkinson (Wiley, 4th Edn 1980).

Despite the authors' stated intentions, I feel that *Concepts and Models* still betrays the unspoken belief of many American chemists that inorganic chemistry is a kind of applied physical chemistry. That is not necessarily a fault, merely a way of looking at the world, but because of it I doubt that the book will be highly recommended in Britain. For me, Porterfield's book is the more attractive and more obviously dedicated to the identity of inorganic chemistry. It is broadly equivalent to Moeller's *Inorganic Chemistry: A Modern Approach* (Wiley, 1982; for review see *Nature* 302, 460; 1983) but because of the criticisms mentioned above I would prefer the latter. Nevertheless, I am glad to have Porterfield and Douglas *et al.* on my shelves — both are thought-provoking and provide new views of familiar landscapes; which is always illuminating. □

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The divine dice keep rolling

D. R. Rosseinsky

Molecular Quantum Mechanics, 2nd Edn.

By P. W. Atkins.

Oxford University Press: 1983. Pp. 471.

Hbk £29.50, \$45; pbk £14.95, \$27.95.

Quantum Chemistry.

By Donald A. McQuarrie.

University Science Books/Oxford

University Press: 1983. Pp. 517.

\$29, £22.50.

Problems in Quantum Chemistry.

By Poul Jørgensen and Jens Oddershede.

Addison-Wesley: 1983. Pp. 286.

\$26.95, £24.30.

OVERHEARD in the corridor of Ancient Languages: "What's new in Classics then, Robin?". "Good Lord, nothing, I hope!". No such exchange about quantum mechanics seems conceivable, yet five decades of elaboration since Pauling and Wilson (four since Eyring, Walter and Kimball) do not obscure the distinct lineage to the three books reviewed here.

Novelty is most evident in the second edition of Atkins's *Molecular Quantum Mechanics*, which is likely to exceed the first in popularity. Most chemistry lecturers (but this is not a book for chemists alone) would probably choose to introduce non-particulate mechanics via Schrödinger rather than Heisenberg, although this may well be out of tune with the customers — students — who these days seem to prefer matrices to differential equations. In accord, Atkins's matrix-based introduction is developed in terms of the powerful operator and group theory methods towards approximate techniques and applications. Here it is that we expect new books to be different, and justice is done to ligand field theory, Hückel's method, bonding, and spectroscopy generally, and much else of what can be treated quantum mechanically. However, Atkins notably omits solids and reaction mechanism. Of the latter he says (*pace* the first edition) "No attention is paid to the theory of chemical reactions which, however fascinating, is still an infant subject". Well, well: theoreticians have a special view of things but such austerity could yet find mitigation in edition three, where adiabatic and nonadiabatic electron transfer might be nominated for honourable mention.

Atkins's book is also of spectacularly fine appearance, with wide margins providing locations for diagrams which thus do not interrupt the text. The separate litho typescript of solutions to the many problems (about 20 at the end of each of the 14 chapters) is by contrast quite acceptable in a companion workbook.

To quote Pauling and Wilson, "A book

written for the reader not adept at mathematics [should] be richer in equations than one intended for the mathematician . . . who can follow a sketchy derivation with ease". By this criterion, all three books are suitable for moderate algebraists, being certainly rich in equations. But McQuarrie has set himself the goal of humanizing the story (as indeed does Atkins, but differently) by the inclusion of brief biographies of the *eminenti*, complete with photographs. The omission of Einstein from this stellar gallery precludes mention of his distaste for a merely probable universe, governed by the deity's dice. Chapter sub-headings tell a story of their own — "Perturbation Theory Expresses the Solution to One Problem in Terms of Another Problem That Has Been Solved Previously"; "Valence-Bond Theory Plus Ionic Terms Are Formally Identical to Molecular-Orbital Theory with Configuration Interaction" — and the whole vehicle of exposition has an attractive facility to it. Matrices only appear halfway through the book, indicative of the level of treatment. The depictions of orbitals are quite nicely illustrative, except for the cotton-wool museum pieces which date back to White in the 1920s. However, omission in the 1980s of ligand fields, even in an introductory text, does seem either too far ahead of or too far behind fashion to be comforting.

Neither Atkins nor McQuarrie uses Coulson's description of the electron as cloud. It is of course formally a probability density, but if the particle is deemed wave- or cloud-like in some aspects, it must be conceptually congruent to believe in a cloud which appears particulate in some of its aspects; the tradition as it has grown derives historically from the greater body of knowledge of particle behaviour than of clouds, and Kimball's notable efforts in the 1950s to restore some even-handedness perhaps deserve perpetuation.

The title of Jørgensen and Oddershede's book, *Problems in Quantum Chemistry*, might excite expectations. It is in fact a misnomer: these are *Student Calculations in a Quantum Mechanics Course*, to be used in conjunction with texts such as the other two reviewed here. Since most student textbooks, in particular Atkins, already contain numerous exercises, the value of such a compendium is to some extent in question; but then the authors do include the transition state, and the case is made. The solutions are well presented and provide a useful educational course not confined to the philosophy of a single text.

The books reviewed here afford the contemporary scientific tyro an access to intellectual avenues which will be invaluable, especially in view of the challenge arising from the present rate and extent of factual accumulation and theoretical development. □

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Rates of exchange

M.L. McGlashan

Basic Chemical Thermodynamics, 3rd Edn.

By E. Brian Smith.

Oxford University Press: 1982. Pp. 160.

Hbk £9.50, \$21.95; pbk £4.95, \$9.95.

Chemical Thermodynamics.

By Peter A. Rock.

University Science Books/Oxford

University Press: 1983. Pp. 548.

\$29, £22.50.

Three Phases of Matter, 2nd Edn.

By Alan J. Walton.

Oxford University Press: 1983. Pp. 482.

Hbk £25, \$49.50; pbk £10.95, \$24.95.

IF CHEMISTRY is about systems in which the composition changes (whether because of "mixing" or of "reaction" is for real systems entirely a matter of convention), and physics is about systems in which the composition is constant, then the first two of these textbooks are of chemistry, and the third is of physics. While Smith and Rock restrict themselves to discussions of equilibrium ("thermodynamic") states, Walton deals not only with equilibrium states but also with the rates of approach to them. All three books are modern in attitude and scope, have been written with modern terminology and symbols, and — at least for the most part — consistently use SI units and the algebra of quantities.

Basic Chemical Thermodynamics is a short, readable well-established introductory textbook suitable for undergraduates during the first half of a degree course. If inevitably it tells much less than the whole of the truth, it manages to avoid telling more than a very few lies. The main change in this third edition is a new final chapter called "The Molecular Basis of Thermodynamics", a concise and welcome addition except for the false implication of its title which ought to have been something more like "The Molecular Basis of that Highly Specialized Small Part of Thermodynamics Concerned with Perfect Gases". It is a pity that Dr Smith did not find a few pages to make it plain, by talking more about real experiments with real apparatus, that thermodynamics is essentially an experimental subject. It is unfortunate, too, that he mars his modern exposition in a few places: by using the same symbol (*P*) sometimes for pressure and sometimes for the ratio (a number) of

Textbook supplement — prices

Where possible both dollar prices pertaining in the United States and sterling prices for the United Kingdom are given in the bibliographical details for each book. Export prices will generally be higher. If a dollar or sterling price is not cited, the book is not available in one of the two countries. However readers should check both price and availability of books before ordering.

two pressures; by recording molar changes as so many kJ rather than as so many $\text{kJ}\cdot\text{mol}^{-1}$; and by hiding so effectively the difference (for example) between an enthalpy change ΔH and the corresponding standard enthalpy change ΔH° . Nevertheless the book can be recommended wholeheartedly to teachers and undergraduates.

In *Chemical Thermodynamics*, Peter Rock evidently sets out to give a complete account of the subject suitable for use throughout an honours degree. Those tempted to adopt the book should first try to find in it the answers to some crucial questions. What exactly is the difference between ΔG and ΔG° for a reaction involving real substances? How could one measure in a real laboratory the entropy change consequent on mixing two real gases at constant temperature and pressure? How can the definition: "The standard state of a gas is the hypothetical ideal gas at one standard atmosphere and the temperature of interest" be used to recover the necessary equation:

$$\begin{aligned}\mu_B^\circ(g, T) &= \mu_B(g, T, p, y_B, y_C, \dots) \\ &- RT \ln(y_B p/p^\circ) \\ &- \int_0^p (V_B - RT/p) dp,\end{aligned}$$

where $\mu_B^\circ(g, T)$ is the standard chemical potential at the temperature T of a gaseous substance B having chemical potential $\mu_B(g, T, p, y_B, y_C, \dots)$ at the temperature T , pressure p , and mole fractions y_B of B, y_C of C etc.; p° is a standard pressure (such as "one standard atmosphere"), and V_B is the partial molar volume of the substance B in the gaseous mixture at T, p, y_B, y_C etc.? Still, the book is a good deal better than most of its many shelf-mates.

Three Phases of Matter is a substantially revised edition ("... around eighty per cent of the English and thirty per cent of the physics has been rewritten") of a textbook which first appeared in 1976. The book is eminently suitable for the introduction of young scientists — whether potentially chemists, biologists or physicists — to the subject that used to be called "Properties of Matter", which, though for no very good reason except that of old-fashioned exposition, had acquired a reputation for being old-fashioned and dull when presented under that name. It contains excellent accounts of the properties of gases and of solids, and an inevitably less satisfying discussion of the properties of liquids (though this section is illuminated by mention of what we have recently learnt from simulation studies). It is a shame, however, that such a book should nowadays contain nothing about the non-analyticity of the free energy of a fluid (or of a solid) in the immediate neighbourhood of a critical point, and the consequences of that. □

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Oceanography: from the deep to the shallows

Henry Charnock

Introductory Dynamical Oceanography, 2nd Edn.

By Stephen Pond and George L. Pickard. Pergamon: 1983. Pp.329. Hbk £25, \$45; pbk £6.95, \$12.50.

Physical Oceanography of Coastal Waters.

By K.F. Bowden. Ellis Horwood: 1983. Pp.302. £27.50, \$68.95.

Environmental Oceanography: An Introduction to the Behaviour of Coastal Waters.

By Tom Beer. Pergamon: 1983. Pp.262. Hbk £15, \$30; pbk £6.50, \$13.

WE HAVE here three new textbooks on physical oceanography, for although Pond and Pickard is an old friend (the first edition appeared in 1978) the present version is much changed in appearance. The smaller page size and the easy-to-read print make a surprising difference: the first edition was reproduced from a typescript, which made the largish pages seem crowded and difficult to read quickly.

There are about 90 more pages but the content is what matters more, of course, and that remains of a high standard — well written, authoritative and managing to convey the fascination to be obtained from applying fluid dynamics to the movement of the ocean. The authors were especially good on scaling and the circumstances in which the full equations of motion can be rationally simplified. Included now is new work on the classical problem of determining ocean currents from observations of the density field and, surprisingly perhaps, a section on eddy-resolving numerical models: the use of such models strains the capacity of the largest computers and their interpretation requires a knowledge and an understanding of the observed characteristics of the ocean. The authors refer to other books for the observational background but a combined treatment at a similarly introductory level would obviously be desirable (though it would take a much larger volume). More illustrations would also help, but that apart it is difficult to see how the book could be better.

Professor Bowden's *Physical Oceanography of Coastal Waters* is based on observation, linked and interpreted by theory expressed in relatively simple mathematics. Intended as a text for undergraduates as well as graduates, it assumes the sort of basic background which Pond and Pickard provide. The latter deal mainly with the deep ocean, but Bowden

has tackled the more taxing problems of the shallower coastal waters in which conditions are complicated by larger tides, more friction, complicated physiography and freshwater run-off.

This text is one of the very few to bridge the gap between the many books dealing with the physics of the deep ocean and the handful discussing estuaries. It gives clear accounts of the major topics — waves, tides and surges, currents, temperature distribution and mixing processes — which are authoritative without being too specialized, though readers who need the details will have to pursue some of the many references. The book is an altogether encouraging start to a new series in marine science.

In *Environmental Oceanography* Tom Beer attempts an even more difficult task, that of introducing the physics of the marine environment to the graduate from a non-scientific background. He intends his book to be used by environmental managers, environmental administrators and students aspiring to such posts (though why they should be drawn from a non-scientific background is not clear).

The book is based on lectures which must themselves have been very stimulating, ranging from fractals to seasickness remedies, and from satellite remote-sensing to a checklist of gear for a coastal environmental investigation. The resulting book seems to me to be less successful, however, containing many interesting sections but lacking integration and coordination. Some statements are incomplete, even plain wrong, and while the errors may not be too serious they make it impossible for this book to be recommended without considerable reservation. Some of my best friends are environmental administrators, and though they may be amused to read that "The Severn bore... is the best known bore in the English-speaking world" they would be mildly insulted to be told that "The symbol π (pi), which represents the number 3.142... is the ratio of the area of any circle to its circumference", and perhaps concerned to read "if 5 tonnes of cyanide is thrown into 100m of water, how long before its concentration drops below the recommended $5\mu\text{g l}^{-1}$? The main difficulty in answering this question is getting all the physical quantities into the same units".

These are all slips, taken out of context. But there are more serious criticisms, for example of the treatment of the tide-generating forces and of the radiation balance of the sea surface which physical oceanographers will find off-putting. If the author could have brought himself to leave out some of the more entertaining but peripheral matter, and to combine the accounts of fundamental material into a coherent whole, his book would have been widely welcomed. □

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A run through quantum mechanics

R. B. Jones

Quantum Mechanics.

By P. C. W. Davies.

Routledge & Kegan Paul: 1984.

Pp.139. Pbk £3.95, \$9.95.

PAUL DAVIES's *Quantum Mechanics*, the first of a series of books entitled *Student Physics*, displays the fast-moving and concise style which has been an attractive feature of his previous science writing. But what has hitherto been a virtue appears on this occasion as a vice. There is insufficient technical detail for the book to be considered as the main text for a first course in quantum mechanics; moreover it does not include enough applications to suit the diverse requirements of most undergraduate physics degree courses.

In just over a hundred pages of text, Professor Davies touches on many standard topics and techniques, including one-dimensional potential well and barrier problems, three-dimensional bound state problems, the simple harmonic oscillator and orbital angular momentum (each done

by both differential equation and operator methods), time-independent and time-dependent perturbation theory and the variational principle. Many particle systems, degenerate perturbation theory and angular momentum addition have about a page each, while three-dimensional scattering and the non-relativistic electron in an electromagnetic field described by a vector potential are not mentioned at all.

Not only is technical detail missing, however, but the laconic discussion of basic principles may mislead the typical student. In connection with the two-slit experiment, saying that "the particle presumably only visits one slit" seems to impute a well-defined position to the particle in the absence of any measurement of its position. In conjunction with the energy-time uncertainty relation, the statement "the law of energy conservation can be slightly violated in quantum mechanics" is apt to confuse students.

In contrast with the miserly text there is, in an appendix, a generous selection of good exercises. The proper use of a sparse but readable book such as this is as a revision text, and for that purpose it will do very well. □

R. B. Jones is a Lecturer in Physics at Queen Mary College, University of London.

Universal arguments

Michael Rowan-Robinson

Introduction to Cosmology.

By Jayant V. Narlikar.

Jones & Bartlett, 20 Park Plaza, Boston, MA: 1983. Pp.470. \$30.

WHAT a pleasant surprise Jayant Narlikar's *Introduction to Cosmology* is. I thought that the book might turn out to be either a mathematical exercise, with little to do with physics and observation, or an example of the conspiracy theory of cosmology. In this theory, of which Fred Hoyle and Geoffrey Burbidge are eloquent advocates, the majority of cosmologists gang up to suppress the mountains of evidence unfavourable to Big Bang cosmology, while at the same time ignoring all the evidence which now, at last, supports the steady state theory.

Both of these fears are unfounded. Narlikar has written a marvellous advanced-level introduction to cosmology in which the mathematical, physical and observational aspects are expounded clearly, simply and in sufficient detail for almost all readers to find themselves drawn into areas they might have thought inaccessible.

● Jayant Narlikar's *Violent Phenomena in the Universe*, first published by Oxford University Press in 1982, has now been issued in paperback. A review of the book appeared in *Nature* 298, 103; 1982. Price of the paperback is £3.95.

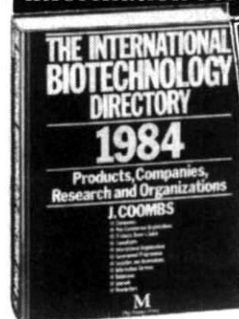
sible. The many hundreds of exercises, complete with hints for their solution, will make this an invaluable course book at final-year undergraduate or postgraduate level, especially for the more theoretically minded students.

A good feature of the book is that some of the weaknesses and incompletenesses of the standard hot Big Bang theory are emphasized (more orthodox types will find this overdone). The more serious of the rival cosmological theories are outlined in some detail and Narlikar is reasonably frank about their shortcomings. He does not pretend, for example, that the steady state theory has much hope of being revived. In outlining the debate about the quasar redshifts, however, he does lay an eccentric emphasis on the non-cosmological interpretation, surely hardly tenable today.

Hardly surprisingly for a book with such a wide scope, it is not up to date in all areas. Neutrinos with non-zero rest-mass are mentioned several times, for example, but not their effect on the galaxy formation problem. There are also one or two small hiccups, like the luminosity of the Sun being given a factor of two too low, and Hubble being cited as having demolished the island universe theory, but these can easily be fixed in the second edition. All in all, this is probably the best cosmology textbook available at this level today. □

Michael Rowan-Robinson is Reader in Astronomy at Queen Mary College, University of London.

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Exotic astrophysics

M. G. Edmunds

Black Holes, White Dwarfs, and Neutron Stars: The Physics of Compact Objects.

By Stuart L. Shapiro and Saul A. Teukolsky.

Wiley: 1983. Pp. 645. Hbk £42.70, \$59.80; pbk £20.45, \$28.70.

WHEN they die, stars leave very interesting corpses. Depending on their mass, single stars either settle down to fade away as dense white dwarfs supported by quantum mechanical degeneracy pressure, or explode as spectacular supernovae. These explosions may sometimes disrupt the star completely, but the compression of matter to very high densities in the cores of the exploding stars frequently produces either a neutron star or, if the core mass is too large, collapse to a black hole.

The dense stellar remnants may also be left in a binary system of stars in which the other member is still undergoing its normal evolution. The exchange of matter from the living star onto the dead can result in a spectacular life-after-death, particularly in the emission of X-rays. A major theoretical effort to understand the structure and properties of compact remnant stars was stimulated during the 1960s and 1970s by two observational developments; one was the launch of rockets and satellites which detected the X-ray sources, the other was the discovery of pulsars.

This impressive volume by Shapiro and Teukolsky, both of whom have been intimately involved with the field, is aimed at introducing the physics of compact objects to advanced undergraduates and first-year graduate students. The wide reach of physics involved — ranging through general relativity, thermodynamics and nuclear, particle, solid-state and plasma physics — inevitably means that much of the underlying theory has to be taken on trust. For example, derivation of astrophysical results reasonably starts by assuming expressions for the Schwarzschild metric and Weinberg-Salam-Glashow weak interaction theory. Likewise a summary of basic astronomy is provided as an appendix, but it is short and again the book will be more understandable to those with some previous knowledge.

Many physical mechanisms in compact objects are still uncertain. We do not know exactly why supernovae explode, although the most likely explanation seems to be simple pressure generated by a shock wave that passes out from the core as it "bounces" back from gravitational collapse when its equation of state stiffens at high densities. The mechanism that generates the intense radiation from pulsars is even more obscure. The authors are commendably brief and non-committal on topics of this kind where not much has been

reliably established. Two important areas of recent research that are well covered are accretion disks — the region from which much of the observed radiation originates as material loses angular momentum and spirals in towards the compact object — and the generation of gravitational waves.

The book is well produced and has many interesting exercises which I found to be full of ideas for use in setting this year's exam questions. My own students beware! It is an excellent introduction to the current state of research — references reach right up to 1982 — that will be of value not only to students but to any physicist or astronomer who does not work directly on the problems of these exotic objects. □

M. G. Edmunds is a Lecturer in the Department of Applied Mathematics and Astronomy at University College, Cardiff.

Making waves

H. Lipson

The Physics of Vibrations and Waves, 3rd Edn.

By H. J. Pain.

Wiley: 1983. Pp. 416. Hbk £18.95, \$35; pbk £7.50, \$13.90.

Vibrations and Waves.

By W. Gough, J. P. G. Richards and R. P. Williams.

Ellis Horwood: 1983. Pp. 278. Hbk £27.50, \$64.95; pbk £7.50.

Waves and Photons: An Introduction to Quantum Optics.

By Edwin Goldin.

Wiley: 1983. Pp. 211. £22.95, \$33.55.

Concepts of Quantum Optics.

By P. L. Knight and L. Allen.

Pergamon: 1983. Pp. 217. Hbk £16.75, \$30; pbk £8.95, \$16.

SOME time ago it was being suggested that physics should not be taught in sections — heat, light and so on — but as a single entity. Topics should be general, coming down to earth, as it were, when obvious applications appeared. Vibrations and waves was mentioned as an appropriate subject for such an approach, but when asked for other examples the proponents of the idea could not think of any. So it is that physics teaching continues very much in its standard form.

If the idea *had* worked, these four books would have served well to illustrate the argument. The first one, the new third edition of Pain's *The Physics of Vibrations and Waves*, is the most elementary. It begins with mechanical systems, which are the easiest for students to envisage, and continues with electric transmission lines, electromagnetic waves, optical waves and finally wave mechanics. Fourier analysis and Fourier transforms are also introduced, and the book contains many well-thought-out problems.

Some of the ideas are, perhaps, oversimplified, however. For example Fermat's principle is stated correctly, but is treated purely as that of least time. Nor is the basis of the principle explained. But my main criticism is that some of the diagrams are inexcusably poor; many of the sine curves are not accurately sinusoidal, and the shapes of parabolic, elliptical and Gaussian curves are unsatisfactory.

Of Gough, Richards and Williams's *Vibrations and Waves* I have no such complaint. It is pitched at about the same level as Pain's book, but is shorter and concentrates on sound and light, giving at one point a detailed discussion of the perception of musical sounds (here it is surprising, given the book's provenance, that no mention is made of the part played by starting transients). Again, there are some excellent problems for the students to test their understanding. Finally, a computer program for the visual presentation of waves is provided. Like Pain, however, these authors devote little space to double refraction in crystals.

The third book, *Waves and Photons*, is less general and more ambitious than the first two, and would certainly not be suitable as an introductory textbook. Like the other authors, Goldin starts off with waves in general but then concentrates on light; he also includes some thought-provoking problems. But the main objective, as stated on the book's jacket, is to try to remove the paradox between wave and quantum optics. The writing is stimulating and intellectually demanding, but it is not easy to say whether the work succeeds; to the experimental physicist like myself, the answer is probably "no", but those who put their faith in mathematics may be satisfied.

The final book, Knight and Allen's *Concepts of Quantum Optics* is quite different again; it would be appreciated by only the very brightest students. The book contains six short chapters, all theoretical, on topics that the authors consider to be of fundamental importance, and these are followed by reproductions of 17 original papers to illustrate points made in the earlier part of the book.

Paper 1 is the first published paper of G. I. Taylor (1909), describing his efforts to see if very weak light could give Young's fringes, even if the probability that two photons could simultaneously pass through the two slits was infinitesimal. (The longest exposure was three months!) J. J. Thomson suggested the experiments, and the fact that perfectly clear fringes were found surprised everyone. It is amusing, in a way, that such a simple experiment as Young's should inspire so much advanced thinking, including the typically Diracian statement that a photon can interfere only with itself. This conclusion, if it can be called such, seems to be negated by Paper 7, by Magyar and Mandel, in which they describe interference fringes obtained from two separate

lasers, albeit with low visibility. But Paper 6, by Pfleeger and Mandel, claims to show that the ideas *are* consistent. What can the poor experimental physicist think?

A further problem is that resulting from the now-famous experiment of Hanbury Brown and Twiss (Paper 14), which, in 1956, introduced the concept of "intensity interference" — at first sight a contradiction in terms. At the time, many people thought the idea preposterous, and were reconciled to it only when Brown and Twiss showed that it produced results. It is now a landmark in optics — as indeed is Paper 2, by Einstein, on the quantum theory of radiation (1921), in which he introduced his ideas of spontaneous and induced (now called stimulated) emission. But perhaps the most exciting paper is the third, by Frisch, written in 1964 to celebrate the seventieth birthday of Professor Oskar Klein of Stockholm. Frisch envisages an intense discussion by five young physicists trying to isolate a single photon. Of course, they do not succeed, even in principle!

So here are four books that have taken us from the foothills to the highest peaks. They have in common the description of what physics is really like — not the acquiring of facts but the pursuit of ideas. It is appropriate to conclude with the quotation by Einstein (1951), on the front page of Knight and Allen's book: "All these fifty years of conscious brooding have brought me no nearer to the answer to the question 'What are light quanta?'" Nowadays every Tom, Dick and Harry thinks he knows it, but he is mistaken!

H. Lipson is Emeritus Professor of Physics at the University of Manchester Institute of Science and Technology.

High in fibre

J.T. Chilwell & S.D. Smith

Optical Waveguide Theory.

By Allan W. Snyder and John D. Love.
Chapman & Hall: 1983. Pp. 734. Hbk £29.95, \$78; pbk £15.95, \$29.95.

ONE of the biggest problems in writing a comprehensive textbook on optical waveguide theory is that of coping with the wealth of mathematical detail which surrounds the subject. In this book, Snyder and Love have found an excellent solution by providing, in their third and final part, supplementary sections "both to augment any deficiencies in mathematical background and to provide a self-consistent and rigorous mathematical approach". Thus in Parts I and II they are able to concentrate on the underlying physical processes while developing the waveguiding concepts.

The result is an authoritative and very readable account — the physical concepts are discussed in parallel with the relevant

mathematics, and where proofs are not given, or only partially given, the steps are outlined in sufficient detail to allow the reader to work them through himself. Although the book is designed for both undergraduate and graduate students, however, a reasonable level of mathematical sophistication is assumed.

In Part I, the authors begin with the planar slab, the ray analysis as applied to multimode guides being developed from the eikonal equation. Where appropriate bound, refracting, tunnelling and tunnelling-refracting rays are considered on circular and non-circular, uniform and non-uniform (axial direction) fibres. Approximations of formulae in the weakly guiding case are given, and worked examples include step, infinitely graded and clad-power law profiles. Absorption, material dispersion, bends and nonuniformities are considered, as well as fibre excitation by collimated and diffuse sources, impulse response, ray transit times and pulse shape/distortion.

In contrast Part II deals with the electromagnetic analysis of optical waveguides, with emphasis on fibres which support only single or a few bound modes. Modal (eigenfunction) methods and to a lesser extent Green's function methods are employed, and as few solutions are tractable much of the analysis is reduced to the weak guidance approximation. Normalized parameters are used throughout and the topics include, among others, sources within fibres, the Gaussian approximation, coupled mode equations, cross talk, plus those covered in Part I (with "ray" replaced by "mode"). The discussion of leaky modes is particularly clear.

The presentation of the book is extremely good: key words and concepts are stressed in italics, key equations are boxed, formulae are collected in tables for easy reference and the text is well illustrated with helpful diagrams. Over 150 problems with complete solutions are integrated with the text.

The title *Optical Waveguide Theory* suggests a larger scope than is actually the case, since the authors concentrate on fibres emphasizing those properties relevant to long range optical communication. Although the physical and mathematical principles are relevant, there is no specific discussion of the channel/rib guide or of components employed in integrated optical systems which are popularly synonymous with optical waveguiding. There are also few references to the literature, but this is no great drawback because of the self-contained development of the subject matter. In all, the book will be of great interest to scientists and engineers working in the optical waveguide field and constitutes a most useful reference volume to this important technological topic. □

John Chilwell is a Research Associate and S. D. Smith a Professor in the Department of Physics, Heriot-Watt University.

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A human T cell-specific cDNA clone encodes a protein having extensive homology to immunoglobulin chains

Yusuke Yanagi, Yasunobu Yoshikai, Kathleen Leggett, Stephen P. Clark, Ingrid Aleksander & Tak W. Mak

The Ontario Cancer Institute, and Department of Medical Biophysics, University of Toronto, 500 Sherbourne Street, Toronto, Ontario, Canada M4X 1K9

We have cloned and sequenced a human mRNA specific for mammalian T-lymphoid cells. The message was found to be expressed in human and murine T lymphoblasts, thymocytes and phytohaemagglutinin-stimulated T lymphocytes. The protein deduced from the cDNA sequence has a molecular weight of 34,938 and shows extensive similarity to the entire length of the variable, joining and constant regions of mammalian immunoglobulin light chains. In addition, the relative positions of the cysteine residues are similar to those of the light chains of murine and human immunoglobulin molecules. These properties suggest that the cDNA clone may correspond to a message that specifies part of the human T-cell receptor.

KNOWLEDGE of the structure of immunoglobulin molecules^{1,2} and the genes³⁻⁶ encoding them has furthered our understanding of the function of B cells and the generation of diversity of immunoglobulin molecules. The findings have led to the conclusion that immunoglobulin molecules are generated by a series of gene rearrangements and RNA splicing events³⁻⁶ that result in polypeptide chains consisting of variable and constant regions¹⁻⁶. These regions can be subdivided into domains held together by interchain and intrachain disulphide bridges situated at the same relative positions¹⁻⁷.

In contrast, very little is known about the generation of T-cell specificity and diversity. Some investigators believe that there is a close relationship between immunoglobulin and the protein that is responsible for specificity of T cells (the T-cell receptor)^{8,9}, while others speculate that entirely different mechanisms or sets of genes are used by T cells¹⁰. During the past year, several laboratories have produced monoclonal antibodies that recognize antigens specific for individual clones of human and murine T cells¹¹⁻¹³. These idiotype proteins may be part of the human or murine T-cell receptor. Although the identification of these tentative T-cell receptor proteins has aided in the definition of their sizes and complexities¹⁴⁻¹⁶, their structures, relationships to immunoglobulins, and biological functions are not clear. Progress in this area has been impeded by the lack of highly purified preparations of these proteins and the absence of molecular clones encoding T cell-specific surface antigens or receptors.

Cloning of T cell-specific mRNA

In an attempt to clone mRNAs specific for T cells, we used the human leukaemic T-cell line, MOLT-3 (ref. 17), which, like other human T-cell lines, is known to express T cell-specific antigens^{18,19}. mRNA was isolated from MOLT-3 cells by oligo(dT)-cellulose column chromatography. Double-stranded cDNAs were generated using the approach described by Land *et al.*²⁰ and inserted into the *Bgl*II site of the vector pFP502EB5 (ref. 21) after selection of 0.5–2-kilobase (kb) fragments by preparative agarose gel electrophoresis. The cDNA was inserted by transfection into *Escherichia coli* strain HB101, and 10,000 independent clones were obtained. A survey of 25 of these chosen at random indicated that the cDNA inserts were of the expected size.

Isolation of T cell-specific clones

To screen for cDNA clones of mRNA expressed either exclusively or preferentially in T cells, the clones were grouped on the basis of their relative levels of expression in MOLT-3 cells and in the human B-cell line, HSC-58 (see Fig. 1 legend). Four of these clones, YT30 (insert size 1.3 kb), YT35 (1.3 kb), YT53 (1.3 kb) and YT76 (0.6 kb), had similar restriction enzyme maps and were all found to hybridize to mRNA from MOLT-3 cells but not to that from HSC-58 cells by Northern gel analysis. A single band of 1.3 kb was detected in MOLT-3 cells (Fig. 1a).

To test whether this message was specific for MOLT-3 or T cells in general we probed RNA from several haematopoietic and non-haematopoietic cell lines and cells. In addition to MOLT-3, two other human T-cell lines (Jurkat and CEM) showed a single 1.3-kb band by Northern gel analysis (Fig. 1a). In contrast, no hybridization was evident with RNA from the following: two other human B-cell lines (RPM1 1788 and RPM1 3638), lymphocytes from a patient with B-cell chronic lymphocytic leukaemia (B-CLL), a human myeloid cell line (K562), a cell line derived from an acute myeloblastic leukaemia (AML) patient (provided by H. Messner), normal human bone marrow cells or a human bladder tumour line (MGHU-1). Additional T-cell populations were also examined, including fresh human thymocytes (provided by E. Gelfand), phytohaemagglutinin (PHA)-stimulated human peripheral blood T cells, and the murine T-cell line RBL-5—RNA from these sources all showed the typical single band in Northern gels (Fig. 1b). The level of hybridization to these RNAs, however, was varied. The thymus cells showed the greatest hybridization, followed by PHA-stimulated T lymphoblasts, and finally the murine T-cell line RBL-5. In contrast, the control RNA from the human B-cell line, HSC-58, and the RNA from a murine myeloma cell line, X63, showed no hybridization.

Taken together, these data show that the cDNA clones YT30, YT35, YT53 and YT76 are similar to RNA from T cells of both human and murine origin, but not to RNA from other haematopoietic or non-haematopoietic sources.

Protein identity of clone YT35

To determine the size of the protein encoded by the mRNA corresponding to the T cell-specific clones, we used the hybrid selection method of Parnes *et al.*²². Total mRNA from MOLT-3

was hybridized to clone YT35 or YT76. After the unattached mRNA was washed off, the annealed mRNA was released and translated *in vitro* using a rabbit reticulocyte lysate system. Figure 2 shows the protein that was synthesized. Two polypeptide bands were detected, one corresponding to a molecular weight (M_r) of ~45,000 (45K) and the other ~30K. The larger

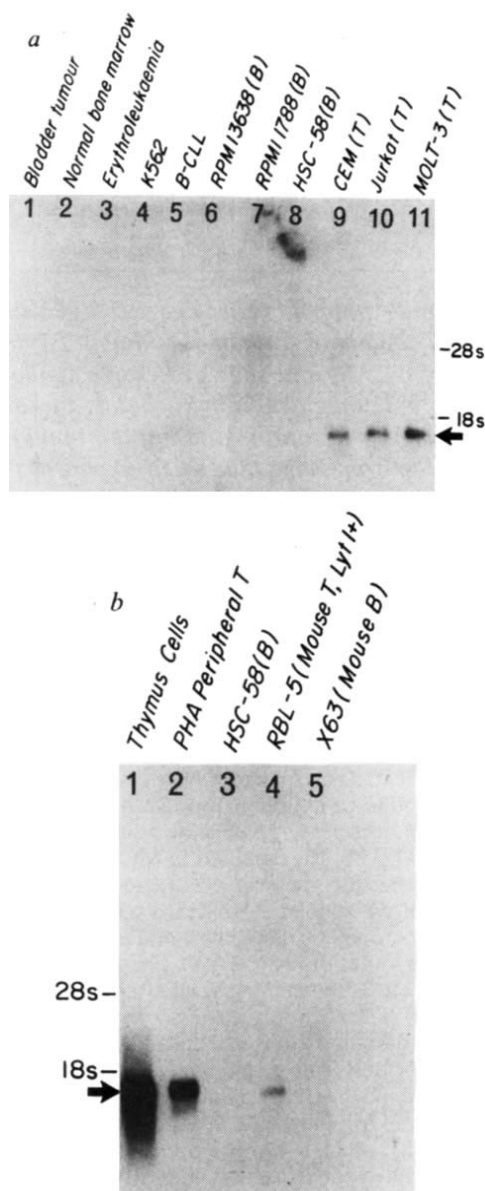


Fig. 1 Northern blot examination of T cell and non-T-cell RNA for hybridization to clone YT35. T cell-specific clones were obtained by a differential hybridization described previously³⁷. RNA was extracted from cells in the presence of guanidine thiocyanate³⁸, treated with glyoxal, then 10 μ g were electrophoresed through 1% agarose in 10 mM sodium phosphate buffer, pH 7.0. Transfer of the RNA to nitrocellulose membranes was carried out as described³⁹. Hybridization was to ³²P-dCTP-labelled, nick-translated clone YT35 (a) or a 0.4-kb *Bgl*III-*Xba*I fragment of YT35 which is internal to the cDNA insert (b). a, RNA is from: lane 1, the human bladder cell line MGHU-1 (provided by Dr I. Tannock); (2) normal human bone marrow; (3) a human erythroleukaemia-like cell line from an AML patient; (4) a human myeloid cell line, K562 (ref. 33); (5) human B-CLL cells; lanes 6-8, human B-cell lines³³; and lanes 9-11, human T-cell lines¹⁸. Cell lines in lanes 2, 3 and 5 were provided by Dr H. Messner. b, RNA is from: lane 1, human thymocytes (provided by Dr E. Gelfand); (2) PHA-stimulated human T-lymphocytes; (3) a human B-cell line³³; (4) a murine T-cell line, RBL5 (provided by Dr R. Miller); and (5) a murine myeloma cell line X63 (provided by Dr N. Hozumi).

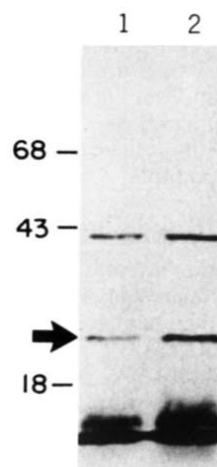
polypeptide was intrinsic to the *in vitro* translation system as it was present in the control without added RNA (data not shown), whereas the 30K polypeptide appeared to be encoded by the mRNA selected by these T cell-specific cDNAs.

Nucleotide and protein sequences of YT35

The nucleotide sequence of clone YT35 was determined using the dideoxy chain terminating inhibitor method of Sanger *et al.*²³; a sequence of 1,151 nucleotides is shown in Fig. 3. Examination of this sequence reveals a long open reading frame with a TGA termination triplet at position 974. A protein that initiates at the first ATG methionine codon in this reading frame (position 38) has a deduced molecular weight of 34,938, which is in general agreement with the results of hybrid selection and *in vitro* translation (Fig. 2). There are stretches of nonpolar and uncharged amino acids adjacent to the N and C terminal, and two possible sites of N-glycosylation (Fig. 3).

To establish whether the deduced amino acid sequence of the T cell-specific clone is similar to any of the immunoglobulin- or T cell-related protein sequences, we surveyed these proteins by diagonal dot-matrix analysis²⁴. The protein sequences deduced from clone YT35 showed a long region of similarity to a murine λ light chain²⁵ (Fig. 4a) and human λ ²⁶ and κ ²⁷ light chains. This homology is more easily recognized when the background is reduced by looking for $\geq 35\%$ similarity over 21 contiguous amino acids. The resulting plots (Fig. 4b-d) show that there are two stretches having high similarity: one near the C-terminal side of the variable region and the other near the N-terminal side of the constant region. Figure 5 shows a direct comparison of these sequences. The highest degree of similarity that was detected in Fig. 4 corresponds to the region surrounding the cysteine residues at positions 111 and 166 in the YT35 sequence. The sequences are quite similar near the cysteine residues at positions 42 and 231 also. Figure 5 also emphasizes the similarity of the amino acid sequences that was detected along the entire light-chain protein in Fig. 4a. Less pronounced homology was also found to the heavy chain of human²⁸ and murine²⁹ immunoglobulin (not shown), but there was no significant similarity to murine H-2 (ref. 30), Thy-1 (ref. 31) or human histocompatibility locus antigens³². The stretch of 249 amino acids that overlaps with the light chains is 33% identical to the murine λ light chain and 38% identical to the constant region. The similarity increases to 59% and 58%, respectively, if one takes into consideration conservative changes. The homology with the constant region of human λ light chain is comparable, with 36% identical amino acids and 61% if conservative changes are taken into account. Note that the protein encoded by clone YT35 is equally similar to the human and

Fig. 2 SDS-polyacrylamide gel electrophoresis of the *in vitro* translation products of the mRNA that hybridizes to clones YT35 and YT76. *In vitro* translation products are from MOLT-3 RNA hybrid selected with clone YT76 (lane 1) or YT35 (lane 2). The positions of molecular weight standards run in neighbouring lanes are marked (in kilodaltons), and the 30K specific protein is indicated with an arrow. Hybrid selection was done according to the procedure of Parnes *et al.*²². Briefly, poly(A)⁺ RNA (isolated by oligo(dT)-cellulose column chromatography) from MOLT-3 cells was annealed to 10 μ g of plasmid YT35 or YT76 that had been bound to nitrocellulose filters. These were extensively washed to remove unbound RNA. Annealed RNA was released by boiling and translated in the presence of ³⁵S-methionine (Amersham) using rabbit reticulocyte lysate *in vitro* translation kits (BRL). The products were electrophoresed on a 10% polyacrylamide-SDS gel⁴⁰ which was treated with En³Hance (NEN) dried and exposed to Kodak XAR-5 film at -70 °C.



MetAspSerTrpThrPheCysCysValSerLeuCysIleLeuValAlaLysHis 18
 CTGGTCTAGAAATATCCACATCTGCTCTCACTCTGCGATGACCTCTGGACCTTCTGCTGTGTCCCTTTGCATCCTGGTAGCGAAGCA
 10 20 30 40 50 60 70 80 90

ThrAspAlaGlyValIleGlnSerProArgHisGluValThrGluMetGlyGlnGluValThrLeuArgCysLysProIleSerGlyHis 48
 TACAGATGCTGGAGTTATCCAGTACACCCGCCATGAGGTGACAGAGATGGGACAAGAAGTGACTCTGAGATGTAAACCAATTTTCAGGCCA
 100 110 120 130 140 150 160 170 180

AsnSerLeuPheTrpTyrArgGlnThrMetMetArgGlyLeuGluLeuIleTyrPheAsnAsnValProIleAspAspSerGly 78
 CAACTCCCTTTTCTGGTACAGACAGACCATGATGCGGGGACTGGAGTTGCTCATTACTTTAACAACAACGTTCCGATAGATGATTTCAGG
 190 200 210 220 230 240 250 260 270

MetProGluAspArgPheSerAlaLysMetProAsnAlaSerPheSerThrLeuLysIleGlnProSerGluProArgAspSerAlaVal 108
 GATGCCCCAGGATCGATTCTCAGCTAAGATGCCCTAATGCATCATCTCCACTCTGAAGATCCAGCCCTCAGAACCCAGGACTCAGCTGT
 280 290 300 310 320 330 340 350 360

TyrPheCysAlaSerSerPheSerThrCysSerAlaAsnTyrGlyTyrThrPheGlySerGlyThrArgLeuThrValValGluAspLeu 138
 GTACTCTGTGCGCAGGTTTCTCGACCTGTTTCGGCTAATGCTACACCTTCGGTTCGGGGACCAAGTTAACCGTTGTAGAGGACCT
 370 380 390 400 410 420 430 440 450

AsnSerValPheProProGluValAlaValPheGluProSerGluAlaGluIleSerHisThrGlnLysAlaThrLeuValCysLeuAla 168
 GAACAAGGTGTTCCACCCGAGGTGCTGTGTTTGGCCATCAGAAGCAGAGATCTCCACACCCAAAGGCCACACTGGTGTGCTGGC
 460 470 480 490 500 510 520 530 540

ThrGlyPhePheProAspHisValGluLeuSerTrpTrpValAsnGlyLysGluValHisSerGlyValSerThrAspProGlnProLeu 198
 CACAGGCTTCTCCCGACCAAGTGGAGCTGAGCTGGTGAATGGGAAGAGGTGCACAGTGGGTGACGACAGACCCGCAAGCCCT
 550 560 570 580 590 600 610 620 630

LysGluGlnProAlaLeuAsnAspSerArgTyrCysLeuSerSerArgLeuArgValSerAlaThrPheTrpGlnAsnProArgAsnHis 228
 CAAGGAGCAGCCCGCTCAATGACTCCAGTACTGCTGAGCAGCCGCTGAGGGTCTCGGCCACCTTCTGGCAGAACCCCGCAACCA
 640 650 660 670 680 690 700 710 720

PheArgCysGlnValGlnPheTyrGlyLeuSerGluAsnAspGluTrpThrGlnAspArgAlaLysProValThrGlnIleValSerAla 258
 CTTCCGCTGTCAAGTCCAGTTCTACGGGCTCTCGGAGAATGACGAGTGGACCCAGGATAGGGCCAAACCCGTCACCCAGATCGTCAGCGC
 730 740 750 760 770 780 790 800 810

GluAlaTrpGlyArgAlaAspCysGlyPheThrSerValSerTyrGlnGlnGlyValLeuSerAlaThrIleLeuTyrGluIleLeuLeu 288
 CGAGGCTGGGTAGAGCAGACTGTGGCTTACCTCGGTGCTCACCAGCAAGGGTCTGTCTGCCACCCTCTCTATGAGATCCTGCT
 820 830 840 850 860 870 880 890 900

GlyLysAlaThrLeuTyrAlaValLeuValSerAlaLeuValLeuMetAlaMetValLysArgLysAspPhe*** 312
 AGGGAAGGCCACCTGTATGCTGTGCTGCTGACGGCCCTGTGTGATGGCCATGGTCAAGAGAAAGGATTCTGAGGGCAGCCCTGGAA
 910 920 930 940 950 960 970 980 990

GTGGAGTTAGGAGCTTTACCCGTCATGGTTCAATACACATTCTCTTTTGGCAGGCTTCTGAAGAGTGCTCTCACCTCTCTGCATC
 1000 1010 1020 1030 1040 1050 1060 1070 1080

CCAATAGATATCCCTATGTGATGCACACCTGCACACTCACGGCTGAAATCTCCCTAACCCAGGGGGAC
 1090 1100 1110 1120 1130 1140 1150

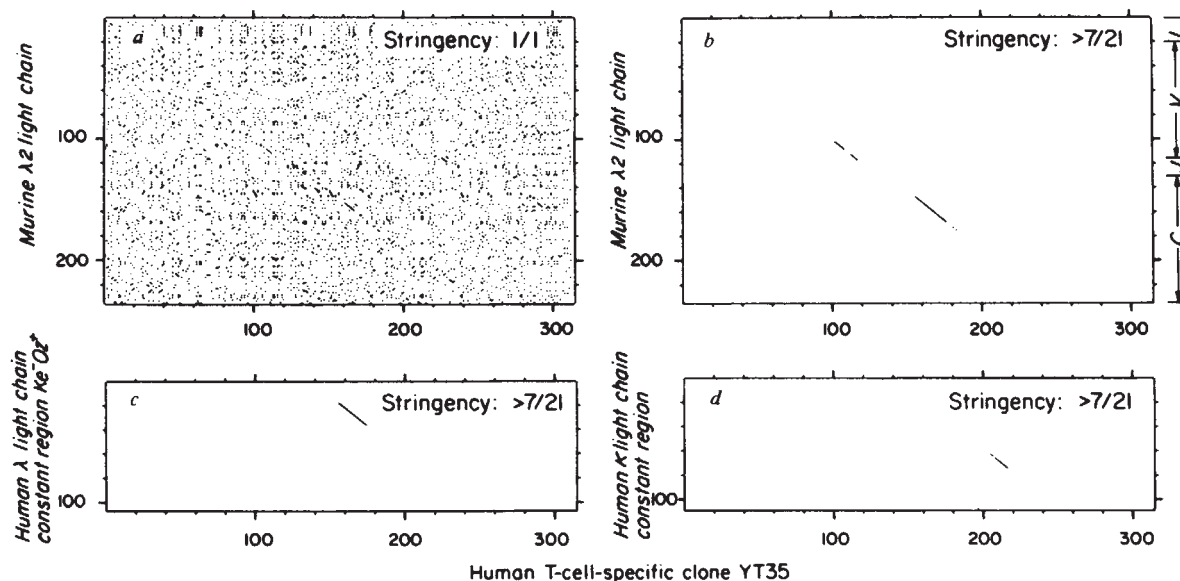
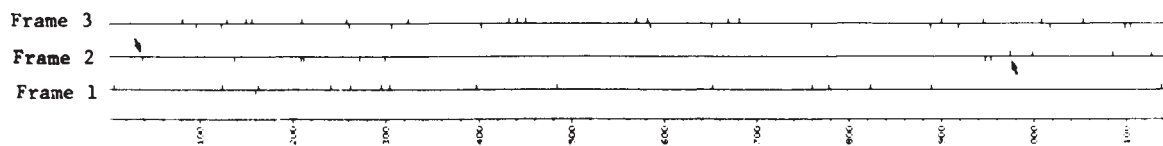


Fig. 4 Diagonal dot-matrix comparison of the deduced amino acid sequence of clone YT35 and the amino acid sequences of murine and human immunoglobulin light chains. The abscissa represents the YT35 sequence while the ordinate represents a murine λ 2 light chain²⁵ (in *a* and *b*), and a human λ ²⁶ (*c*) or κ ²⁷ (*d*) light chain. In *a*, wherever the amino acids were the same at the corresponding *x* and *y* positions a dot was plotted, that is, the stringency was set to 1 out of 1. In the remaining panels the background was reduced by plotting a dot only when >7 within a span of 21 consecutive positions were identical, that is, at $\geq 35\%$ homology. L, leader peptide; V, variable region; J, joining segment; C, constant region.

1	MDSWTFCCVS---LCLILVAKHTDAGVIQ-SPRHEVTEMGQEVTLRCKPISG---HNSLFW-YRQT-MMRGLELLIYFNNN-V	YT35
	* * * * * * * * * * * * * * * * * * * * * * * * *	
-19	MA-WTSLILSLALCS-GASSQ-AVVTQESA---LTTSPGGTVILTRCSSTGAVTTSNYANWIEQKPDHLFTGLIGGTSNRA	Murine λ light chain
	-- Signal Peptide -- -- HVR1 -- -- HVR2 --	
1		Human λ light chain
		* * * * *
73	PIDDSGMPEDRFSAKMPNASFSTLKIQPSEPRDSAVYFCASSFSTCSANYGYTFGSGTRLTVVEDLN-KVFPPEVAVFEP	YT35
	* * * * * * * * * * * * * * * * * * * * * * * * *	
58	P----GVP-VYRSGSLIGDKAA-LTIITGAQTEDDAMYFCALWF----RNH-FVPGGGTKVTVL--GQPKST-PTLTWFFPPS	Murine λ light chain
	-- HVR3 -- -- J Region --	
14	SEELQA-NKATLVCLISDFYPGAVTVAMKADGSPVKAGVETT-KPSK-QS--N-NKYAASSYL--SLTPEQ-WKSHRSYS	Human λ light chain
	* * * * * * * * * * * * * * * * * * * * * * * * *	
153	EAEISHTQKATLVCLATGFFPDHVELSMWVNGKEYVHSGVSTDPQLKEQPALNDSIRYCLSSRLRVSAFTWQNPNRH--FRC	YT35
	* * * * * * * * * * * * * * * * * * * * * * * * *	
125	SEEL-KENKATLVCLISNFPSPSGVTVAWKANGTPTIQGVDTN-NPTKE--G---NKFMASSFLHL--TSDQ-WRSHNSFTC	Murine λ light chain
86	QVTHEGSTVEKTVAPTECS Human λ light chain	
	* * * * * * * * * *	
232	QVQFYGLS-ENDEWTQDRAKPVYTIQVSAEWAGRADCGFTSVSYQQGVLSATILYEILLGKATLYAVLVSAVLVMAWVKRKDF	YT35
	* * * * * * * * * *	
197	QVTHEGDTVEKSLSPAEL Murine λ light chain	

glycine (G), isoleucine (I), leucine (L), methionine (M), proline (P), valine (V), tryptophan (W) and the murine λ light chain are marked. HVR, hypervariable region.

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Isolation of cDNA clones encoding T cell-specific membrane-associated proteins

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Of 10 distinct cloned DNA copies of mRNAs expressed in T lymphocytes but not in B lymphocytes and associated with membrane-bound polysomes, one hybridizes to a region of the genome that has rearranged in a T-cell lymphoma and several T-cell hybridomas. These characteristics suggest that it encodes one chain of the elusive antigen receptor on the surface of T lymphocytes.

T LYMPHOCYTES, like B lymphocytes, are capable of recognizing a wide range of different antigens^{1–3}. As with B cells, the ability to recognize a given antigen is fixed in any particular clonal line of T cells. However, unlike B cells, T cells appear to recognize antigens in combination with self major histocompatibility (MHC) determinants^{4–6}. In view of the similarities to B cells, early ideas as to how T cells recognize antigens centred on the use of either entire antibody molecules⁷ or at least some of the separately encoded (in the germ-line genome) segments that make up the antigen-binding sites of immunoglobulin heavy and light chains^{8–12}. But despite early reports that antibodies against immunoglobulin antigen-binding sites can react with T cells^{13–15} and recognize a target closely linked to the immunoglobulin heavy-chain locus^{16,17}, attempts to demonstrate an involvement of immunoglobulins in T-cell antigen recognition have proved consistently negative^{8–12}.

More recent investigations have taken a route largely independent of the antibody models and have succeeded in raising at first antisera¹⁸ and then monoclonal antibodies^{19–22} that specifically recognize particular T-cell lines or hybridomas. Some of these antibodies have been shown either to inhibit^{20–22} or stimulate¹⁸ the response of a cell in a clone-specific fashion. Several groups have used these antibodies to immunoprecipitate disulphide-linked heterodimers, composed of two distinct glycoproteins of molecular weights 37,000–50,000 (refs 19–22), both of which appear to have variable and constant regions^{23,24}.

We have taken a different approach to this problem and have attempted to use the techniques of molecular genetics to isolate from a series of antigen-specific, MHC-restricted T helper hybridomas^{25,26} the genes that encode their antigen receptors. Our approach was based on the following assumptions about the nature of the T-cell receptor genes: (1) That they should be expressed in T cells but not in B cells. (2) That the mRNAs for the T-cell receptor proteins should be found on membrane-bound polysomes, as one would expect the nascent receptor

polypeptides to attach to the endoplasmic reticulum by a leader peptide, or signal sequence²⁷. (3) That like immunoglobulin genes those that encode the T-cell receptor, proteins should be rearranged in T cells as a mechanism of generating diversity and consequently increasing the antigen-recognition repertoire. (4) That like immunoglobulin genes they should have constant regions (as they presumably share at least some functions) and variable regions, would confer the antigen-binding specificity.

An experimental strategy could be developed on the basis of these assumptions, as B and T cells differ in only a small fraction of their gene expression (~2%, or 200–300 different sequences²⁸; M.M.D. and W. E. Paul, manuscript in preparation) and only a small proportion of lymphocyte mRNAs appear to be in the membrane-bound polysomal fraction (~3%)²⁹. Thus, by synthesizing ³²P-labelled DNA copies (cDNAs) of the membrane-bound polysomal RNA of antigen-specific T cells and removing by RNA hybridization those sequences also expressed in B cells, one should be left with a specific probe representing a very small fraction of total T-cell gene expression and likely to include copies of the mRNAs that encode the T-cell receptor. This probe can then be used to screen a library of cloned cDNAs³⁰, and the clones thus identified could be used to look for somatic gene rearrangements in T cells. Further restriction mapping and sequence analysis of the cDNA clones derived from different T cells might reveal variable and constant regions. We have carried through this strategy, and have managed to obtain a cDNA clone (TM86) representing mRNA that is expressed specifically in T cells, found in the membrane-bound polysomal fraction and encoded by genomic DNA that has rearranged in T cells.

Analysis of T-cell mRNA

The number of different species in a given mRNA population can be estimated from an analysis of the kinetics of cDNA-mRNA hybridization reactions³¹. In this case, labelled cDNA was synthesized from cytoplasmic poly(A)⁺ RNA of the T helper hybridoma M12 (ref. 26), the RNA template was removed and the cDNA hybridized to the original RNA. The resulting C₀t

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Table 1 Analysis of C_0t curves

Labelled cDNAs	Component	Fraction	$C_0t_{1/2}$	$C_0t_{1/2}$ Pure	No. of species	mRNAs per cell
Cytoplasmic	1	48.6%	0.52	0.18	130	1,050
	2	51.4%	47.4	17.7	12,900	12
Free	1	56.8%	0.12	0.048	—	—
	2	43.2%	25.6	7.77	—	—
Membrane-bound	1	67.6%	0.12	0.058	—	—
	2	32.4%	15.7	3.8	—	—
MB T_H^* -B	1	41.7%	1.25	0.25	1	—
	2	58.3%	92.8	26.0	70	—

Reaction	Double-stranded	Single-stranded	
1	91.3%	8.7%	($T_{M12}^* - B_{L10A}$)
2	32.5%	67.7%	($T_{M12}^* - B_{L10A}$)
3	44.4%	55.6%	($T_{M12}^* + T_{M12}$)

Least-squares fit analysis³² of the reactions shown in Fig. 1. The curves were fitted to two components with root mean square (r.m.s.) errors of 0.03–0.05. The fractions were normalized to 100% and both uncorrected and 'pure' $C_0t_{1/2}$ values are shown. The number of species was determined using the corrected $C_0t_{1/2}$ values and an average mRNA size of 2,000 nucleotides²⁹. Purified ovalbumin mRNA (given by G. Stanley McKnight) was used as a kinetic standard. The calculation of number of mRNAs per cell is based on the average of 300,000 cytoplasmic poly(A)⁺ RNAs we observe in most T hybridomas in log phase growth. The MB T_H^* -B analysis draws on the data in Fig. 1b. As we reproducibly find that the remaining B cell-reactive material consists largely of small, very slowly reacting cDNA which introduces a physiologically irrelevant slow component into the C_0t analysis (M. M. D. and W. E. Paul, manuscript in preparation), we subtracted the background curve directly from the homologous curve and computer fitted the difference. In this case, the L10A reaction with the MB T_H^* -B is subtracted from the M12 reaction and fitted as shown in this table. The second part of the table summarizes the cDNA subtraction, with two successive subtractive hybridizations with B-cell (L10A) RNA follow by a back reaction with homologous (M12) RNA to remove unreactable sequences. The repetitive yield is 50–65% for each round of reaction and it is not selective for single-stranded or double-stranded nucleic acid (M. M. D. and W. E. Paul, manuscript in preparation). The single-stranded fraction is carried through to the next step from both reactions 1 and 2, and the double-stranded fraction of reaction 3 is the material analysed in Fig. 1b and which represents 2.6% of input membrane-bound polysomal cDNA, normalizing for yield losses. As the analytical reactions (Fig. 1b) indicate that 61% of this material represents T cell-specific sequences, 1.6% (2.6×0.61) of the initial cDNA is unique to T cells. As the membrane-bound RNA represents only 15% of the total, this value is further reduced by this fraction ($1.6\% \times 0.15$) to yield 0.24% of the mass of the cytoplasmic mRNA. As perhaps an equal amount of these same sequences are found in the free polysomal fraction (see text), the value of 0.24% must be revised upwards to 0.5%. This number is then divided into abundant and rare fractions as indicated in the table and the number of sequences of average size derived (~ 1 for component 1 and 70 for component 2).

(or R_0t) curve was computer-fitted by the least-squares program of Pearson, Davidson and Britten³² and is shown in Fig. 1a. The analysis of this curve, presented in Table 1, indicates that there are about 13,000 different mRNA species in this cell line, most of which are in the rare abundance class (component 2 in Table 1) at about 12 molecules per cell. These findings are typical of a variety of *in vitro* cultured cell lines³³ although somewhat higher than the 8,000 different species seen in a murine plasma cell line²⁹. Also shown in Fig. 1a are the reactions of cytoplasmic poly(A)⁺ RNA with labelled cDNAs prepared from membrane-bound and free polysomal fractions of RNA, using the procedure of Mechler and Rabbitts²⁹. Although a striking feature of that report was the detection of virtually no rare mRNA species in the membrane-bound fraction of a plasmacytoma, the analysis of the data presented here (Table 1)

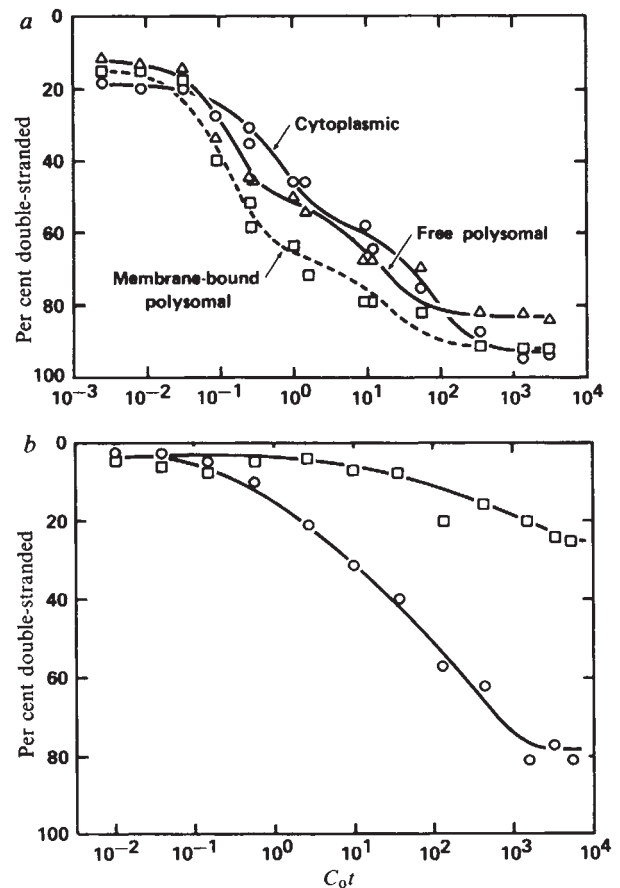


Fig. 1 Kinetic hybridization analysis of T-helper hybridoma mRNAs. *a*, Hybridization to an excess of cytoplasmic poly(A)⁺ RNA of: ○, total cytoplasmic cDNA; △, free polysomal cDNA; and □, membrane-bound cDNA. *b*, Hybridization of the MB T_H^* -B_{L10A} probe to M12 cytoplasmic poly(A)⁺ RNA (○) and hybridization of the same probe to L10A poly(A)⁺ cytoplasmic RNA (□).

Methods: Cytoplasmic poly(A)⁺ RNA was prepared as described⁴⁶ from an *in vitro* cultured hybridoma line (M12). Membrane-bound and free polysomal poly(A)⁺ RNAs were prepared from these cells by the procedure of Melcher and Rabbitts²⁹. ³²P-labelled cDNA was synthesized from these RNAs using oligo(dT) and reverse transcriptase³⁰. cDNAs were depleted of template RNA by base hydrolysis and reacted in 0.5 M phosphate buffer⁴⁷, 0.1% SDS, 5 mM EDTA in sealed glass capillaries with an excess of cytoplasmic poly(A)⁺ RNA. Reactions were assayed by hydroxyapatite chromatography and analysed as described in Table 1. C_0t values as given are normalized for the effect of the high salt⁴⁷.

clearly indicates the existence of a rare abundance class component of the equivalent fraction in M12 cells.

When cDNA from membrane-bound polysomal RNA is repeatedly hybridized to B-cell mRNA and fractionated on hydroxyapatite (Table 1), the non-hybridizing DNA consists of 2.6% of the input cDNA, and the reactions shown in Fig. 1b (and analysed in Table 1) indicate that 61% of this fraction is T cell-specific and divided as shown between rare and moderately abundant classes (components 2 and 1 respectively, the $C_0t_{1/2}$ 'pures' of which are almost indistinguishable from those of cytoplasmic cDNA). Although only 15% of the polyribosomal RNA was membrane-bound in these cells, the T cell-specific genes were found to be as prevalent in the free polysomal as in the membrane-bound fractions of RNA. We therefore estimate that the pool of membrane-bound RNA derives from $\sim 30\%$

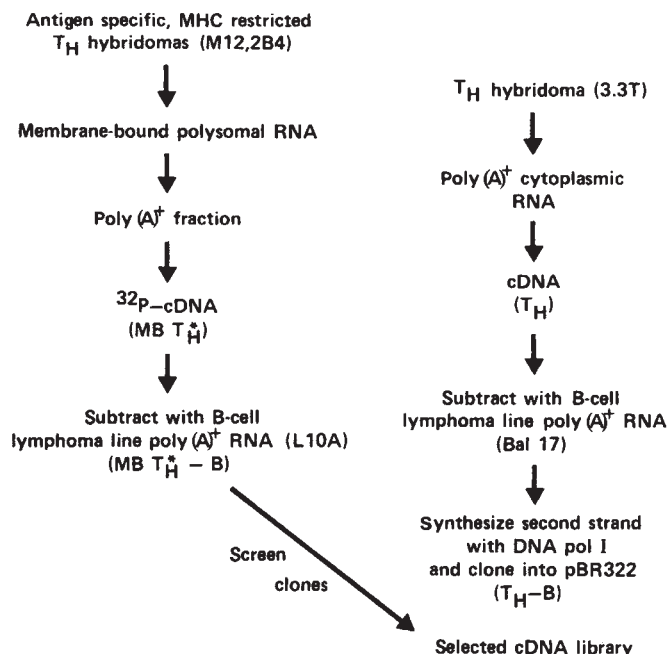


Fig. 2 Strategy of clone isolation. ^{32}P -labelled cDNA was synthesized from membrane-bound polysomal RNA of T_H hybridomas and subtracted with B-cell mRNA (L10A). These probes were then used to screen a selected cDNA library (T_H -B) constructed as described³⁰ from another T_H hybridoma/B cell combination.

of the total polysomal RNA. Thus, the subtracted cDNA ($\text{MBT}_{\text{M12}}^* - \text{B}_{\text{L10A}}$) represents $\sim 0.5\%$ of the input cDNA and ~ 70 different species (see Table 1 legend). This latter number could actually be an overestimate for the following reasons: (1) Rare mRNAs have, in several cases^{34,35}, been shown to be larger than the average mRNA. (2) A certain fraction (0.2–0.4%) of the cDNA differences between any two mRNA populations are not reproducibly expressed in other cells of the same type, indicating a certain background level of random gene expression or loss of expression (M.M.D. and S.M.H., unpublished observation). (3) The use of total cytoplasmic RNA rather than polysomal may introduce a significant number of non-translated sequences into the estimates.

cDNA clone isolation

The strategy used to isolate cDNA clones representing membrane-bound mRNAs expressed in T but not in B cells is shown in Fig. 2. Membrane-bound T helper (T_H) cell cDNA probes were subtracted with B-cell mRNA and used to screen a cDNA library that was itself the product of a T_H -B cell reaction, and which was constructed as described previously^{30,36} for a B cell-specific library³⁰. The T_H -B library was 20-fold enriched for T cell-specific sequences as judged by the fact that 95% of the mass of the cDNA was removed in the subtraction (at the hydroxyapatite stage). As indicated in Fig. 2 the library of 5,000 selected clones was screened and rescreened by standard procedures^{37,38} using the $\text{MBT}_{\text{2B4}}^* - \text{B}_{\text{L10A}}$ probe. Thirty-five clones were screened and rescreened positive (TM clones). In order to determine which clones were derived from the same mRNA species and which were different, as well as to remove any false

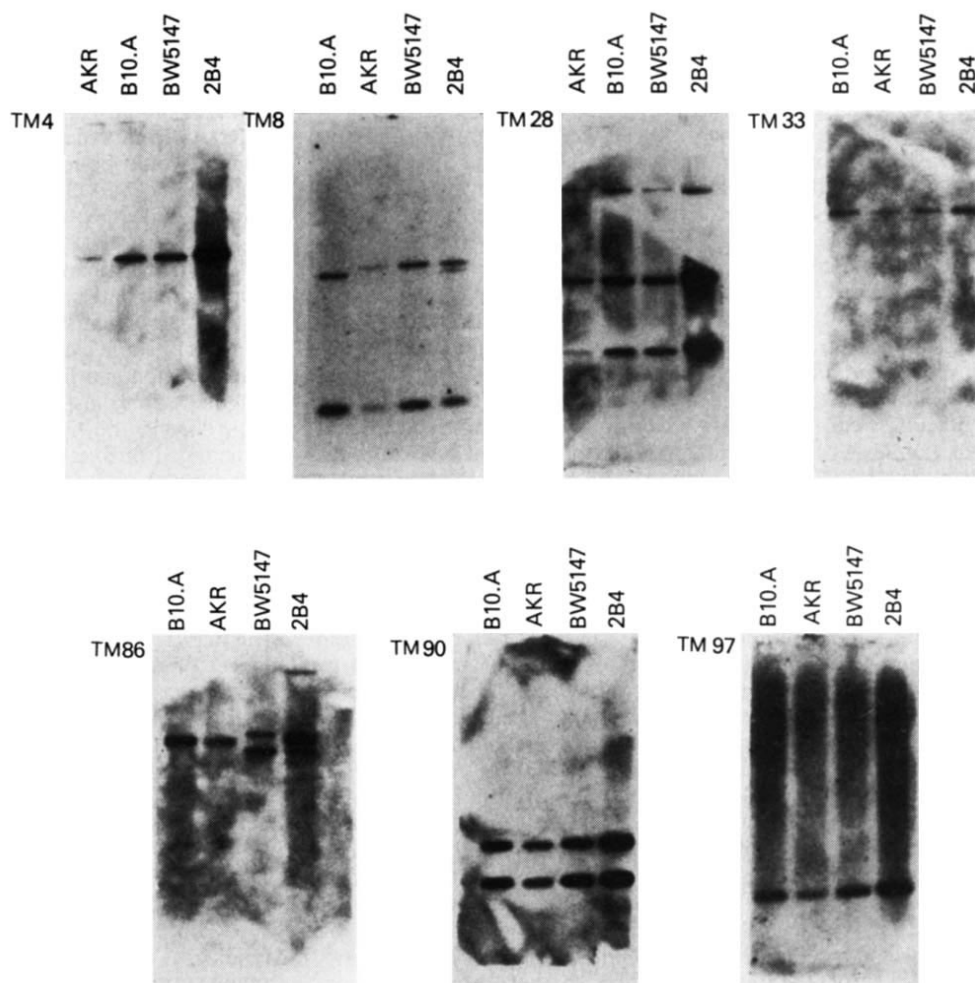


Fig. 3 Southern blot analysis of TM clones. ^{32}P -labelled inserts from the seven clones indicated were hybridized to Southern blots containing DNA from liver cells of strains B10.A and AKR, and DNA from BW5147 and the T_H hybridoma 2B4. The DNAs were prepared by standard methods⁴⁸, digested with the restriction enzyme *Pvu*II, electrophoresed through 0.9% agarose and blotted onto nitrocellulose. Hybridization was in 50% formamide, $5\times\text{SSPE}$ ⁴⁸ at 42°C . Washing was done in $2\times\text{SSPE}$ at 55°C .

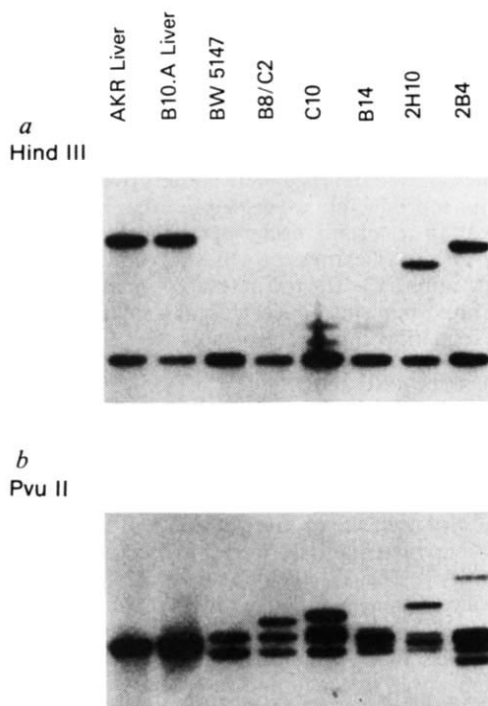


Fig. 4 Analysis of other T_H hybrids for rearrangement of TM86. In this case, four additional antigen-specific hybridoma lines were examined for rearrangement using the TM86 probe and the procedures outlined in Fig. 3 legend. B8/C2 and B14 are T_H hybrids specific for insulin and C10 is a T_H hybrid for lysozyme⁴⁹.

positives, we ³²P-labelled by nick-translation each positive clone to make labelled probes which were then hybridized to representative Northern blots. Five of the positive clones were reactive with B-cell mRNA, and the remaining 30 fell into one of 10 distinct patterns of mRNA size and expression. One of these 10 different clones cross-hybridized strongly with and gave a Northern blot pattern superimposable on a rat Thy-1 cDNA clone isolated by Silver and colleagues³⁹. As Thy-1 is a classical T-cell membrane antigen not expressed on B cells, the fact that it is one of the genes isolated in this small set is an important internal control.

Our procedure for obtaining cDNAs of membrane-bound, T cell-specific mRNAs should have enriched for particular sequences to such an extent that even very low abundance mRNAs are represented at levels comparable to those of abundant mRNAs in the original population. This should overcome the difficulties earlier workers have found in detecting low abundance mRNAs^{40,41}. In fact, hybridization of the inserts of the less abundant TM clones to the 3.3T-selected cDNA library in at least two cases indicates that we have been able to isolate DNA copies of mRNAs represented by only 1 in 100,000 clones in the unselected library.

Somatic gene rearrangements

To determine whether any of the seven TM clones which, on Northern blotting, were shown to represent mRNAs large enough to encode T-cell receptor proteins¹⁹⁻²², had been copied from mRNAs encoded by genes that, like immunoglobulin genes, rearrange somatically, we prepared labelled probes from them which we hybridized to Southern blots of genomic DNA from the thymoma BW5147 (from the mouse strain AKR), AKR liver cells, the antigen-specific T-cell line 2B4 (a fusion of T cells from B10.A mice with BW5147) and B10.A liver cells. The autoradiograms from Southern blots are shown in Fig. 3. Except for the restriction polymorphism between AKR and B10.A seen with TM8 (Thy-1), the patterns of hybridization with each clone were identical for all the sources of DNA except in the case of TM86 (Fig. 3). Clearly, there was a strikingly

different pattern of *Pvu*II fragments that hybridized to the clone from either BW5147 or 2B4 compared with liver DNA from either of the parental strains. The clones surveyed here were also hybridized to *Eco*RI and *Hind*III digests of genomic DNA, and in each case only TM86 showed a significant difference between the T-cell DNAs and liver DNAs.

It would be expected that genomic rearrangements of a receptor gene should be different for T cells of different antigen specificities. To test this possibility, genomic blots consisting of DNA from five antigen-specific T-cell hybridomas were hybridized with a nick-translated insert from clone TM86. The results, shown in Fig. 4, demonstrate that DNA from each of the antigen-specific T cells gives a different pattern. Three different B-cell lymphoma DNAs give patterns identical to that of the liver (data not shown), indicating that this type of rearrangement may only take place in T cells. It is also significant that no restriction fragment length polymorphisms can be detected with TM86 between the two strains for which data are shown here or in the three other strains that we have examined, either with the *Pvu*II enzyme or several others. This diminishes the possibility that the rearrangements could be due to the presence of a viral sequence since one would expect a highly unstable integrated viral sequence to be changing at least as dramatically in evolutionary time.

Discussion

As previously demonstrated^{43,44} the techniques used in this report provide a general means of isolating sets of genes that are particularly important to the differentiated state of a cell. This set should include at least a portion of the genes required for the specialized functions of the cell, as well as genes involved in regulation and cellular differentiation. As the set of DNA clones obtained is very small, elaborate screening procedures can be used to further define them. In cases where the target population is very small (~0.5% for the set of genes examined here) the enrichment of the relevant probe (~ $\times 200$) is sufficient to allow the isolation of very rare genes. For the studies reported here, we were able to screen a small set of cDNA clones for possible somatic rearrangement of the genes encoding the mRNAs from which they were derived. Such a screening procedure would be much too laborious if used to screen a large number of cDNA clones. An extensive characterization of TM86 and related cDNA clones is presented in the accompanying report⁴².

The striking characteristic of TM86 is that the genes undergo somatic rearrangement in T cells but not B cells. The pattern of *Pvu*II fragments from liver DNA which hybridize to TM86 consists of three closely spaced bands of 5.2–6.5 kilobases (kb). The T lymphoma BW5147 has retained none of the germ-line fragments, and instead shows a pattern of two bands different from any in the liver DNA. We conclude that BW5147 has rearranged one or both of its gene copies; if it has rearranged only one, then it must have lost the other. Consistent with the notion that TM86 represents one chain of the T-cell receptor, each of the different antigen-specific T-cell hybridomas has one or more new homologous restriction fragments. Interestingly, each of the antigen-specific T-cell hybridomas has the same two fragments as BW5147, but has lost various of the germ-line fragments. Considering that immunoglobulin genes are the target of oncogene translocations in B lymphomas and plasmacytomas⁴⁵, it is attractive to speculate that the maintenance of the rearranged chromosomes of BW5147 reflects the translocation of an oncogene to TM86.

We thank E. Heber-Katz, D. Hansburg, L. Samuelson and R. Schwartz for providing the T-helper hybridoma lines used here; J. Kim and R. Asofsky for the B-cell lymphoma lines; D. Margulies and R. Germain for some of the cell line and liver DNAs; J. Silver for the rat Thy1 cDNA clone; and G. Stanley McKnight for the generous gift of purified ovalbumin mRNA. We thank R. Schaller, J. Rothbard and N. Gascoigne for critical reading of the manuscript. We also thank W. E. Paul for continual support and encouragement.

Received 25 January; accepted 31 January 1984.

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Sequence relationships between putative T-cell receptor polypeptides and immunoglobulins

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Comparison of the sequence of a cloned T cell-specific cDNA with those of cross-reacting cloned cDNAs isolated from a thymocyte library indicates the presence of variable, constant and joining regions remarkably similar in size and sequence to those encoding immunoglobulin proteins. Together with the evidence for somatic gene rearrangements reported in the accompanying paper, this strongly suggests that the TM86 cDNA clone encodes one chain of the T-cell receptor for antigen.

IN THE preceding paper¹ we reported the isolation of a cDNA clone, TM86, which represents a species of mRNA that is expressed in T cells but not in B cells, encodes a membrane-associated protein, and is rearranged in T cells. We report here our further analysis by nucleotide sequencing of clone TM86, and also of several cross-reacting clones isolated from a thymocyte cDNA library. Our data reveal a remarkable resemblance between the amino acid sequences of the proteins encoded by the mRNAs represented by the TM86 and related cDNA clones and the immunoglobulin proteins of B cells.

Immunoglobulin proteins have a characteristic primary and secondary structure made up of a series of intrachain disulphide-bonded domains (as reviewed in ref. 2). Both heavy and light immunoglobulin chains begin with a leader peptide of 17-29 residues, followed by a variable region of 94-97 residues then a joining region of 13-17 residues (in heavy chains the variable and joining regions are separated by short 'diversity' elements of 1-14 residues). These elements are encoded separately in the germ-line genome by gene segments that are brought together during B-cell ontogeny³⁻⁶ to generate an enormous range of different antibody genes. The domains of the constant regions

of immunoglobulins (of variable number depending on polypeptide chain type and class) are encoded by separate exons⁷, but they do not appear to rearrange during development. The sequences of our T cell-specific cDNA clones has revealed a similar arrangement of constant and variable regions, strongly suggesting that the mRNAs that they represent encode polypeptide components of the T-cell antigen receptor.

Characterization of cDNA clones

In view of the evidence presented in the preceding paper that the region of the genome represented by cDNA clone TM86 is rearranged in T cells, we sought to isolate cDNAs representative of independent rearrangement events by screening a thymocyte cDNA library (provided by C. Benoist) with TM86 using standard conditions⁸. Positive clones were subcloned into the *EcoRI* site of plasmid pUC9 (ref. 9). Partial restriction maps and the sequencing strategies¹⁰ are shown in Fig. 1 for TM86 and the three thymus-derived clones 86T1, 86T3 and 86T5. The 3' termini of the 86T series of clones are all identical, as each clone has an internal *EcoRI* site in that region, and the variation in position of the 5' termini is presumably due to random chain termination during library construction.

As the largest mRNA detected on Northern blots with TM86 has a size of ~1,300 bases¹, by subtracting a poly(A) tail of

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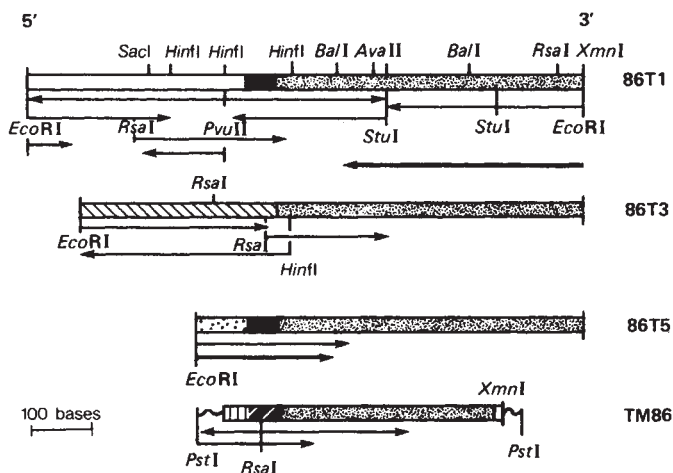


Fig. 1 cDNA clone sequencing strategy and regions of similarity. The TM86 cDNA clone was obtained as described in the accompanying article¹, and the insert excised by *Pst*I from the plasmid vector. The insert was ³²P-labelled by nick-translation⁸ and used to screen a λ gt-10 cDNA library constructed from total thymocyte poly(A)⁺ RNA from young, BALB/c mice (T. Huynh and R. W. Davis, unpublished protocol). Shaded areas indicate major similarities between the different cDNA clones. The 5'→3' orientation was deduced from the large open reading frame of 86T1. Restriction sites indicated for 86T1 are shared within regions having the same shading on the cDNA clones. Sequencing was by the procedure of Maxam and Gilbert¹⁰, with thick arrows representing 3'-end labelling and thin arrows 5'-end labelling.

150–250 bases we would expect a cDNA insert size of around 1,100 bases. Thus, the 938-base insert of clone 86T1 should contain most of the sequence of the mRNA it represents. The insert of 86T1 was completely sequenced and compared with partial sequences of the other clones (see Fig. 1). As implied by Fig. 1, the 3' end of TM86 appears to be different from those of the 86T series, which may have a bearing on thymic maturation and will be the subject of a separate report.

The nucleotide sequences and predicted amino acid sequences of the four different cDNA clones are given in Fig. 2. It can be seen that an open reading frame exists throughout the entire 86T1 insert, beginning with a methionine codon 22 nucleotides from the 5' end and extending for 306 amino acid residues through the 3' *Eco*RI site. The positions of cysteine residues and five potential carbohydrate attachment sites are indicated. Several interesting points are evident from these sequences: (1) Although all of the clones have identical 3' end sequences (except where noted above for TM86) the 5' end sequences are all very different. We have recently sequenced the entire 'constant' region of 86T3 (D. Becker and M.M.D., unpublished results) and it differs from the sequence of 86T1 by only a single nucleotide. This arrangement of constant and variable regions is similar to that observed with immunoglobulin cDNA clones. (2) Immediately following the methionine initiation codon there is a stretch of hydrophobic residues corresponding to the expected leader peptide (the sequence Leu-Leu-Leu is common in immunoglobulin κ light-chain leader peptides). (3) 86T1 and 86T5 share a 16-residue sequence between the constant and variable regions that is not found in the sequences of 86T3 and TM86, suggesting the existence of an independently assorting *J*-like region. (4) The positions of cysteine, and other, residues is similar to that of immunoglobulin and related molecules. (5) Clone 86T3 appears to be derived from a non-functional mRNA as it has stop codons in every reading frame.

Similarity to immunoglobulins

To identify possible evolutionary relationships between the sequence of cDNA clone 86T1 and other known sequences, we searched the Dayhoff protein sequence data bank using the

rapid comparison programs of Wilbur and Lipman¹¹. From the Dayhoff bank of ~2,300 sequences, 25 were found to have a similarity to the 86T1 sequence greater than, or equal to, five standard deviations from the mean. Of these 25 sequences, 24 were immunoglobulin sequences and one was of a class II human major histocompatibility molecule (HLA-DC1 α). Furthermore, it was found that the apparent variable region of 86T1 matched with variable regions of immunoglobulins and the constant region with immunoglobulin constant regions (and in one case with the second domain of HLA-DC1 α , which has previously been shown to have a sequence related to those of immunoglobulins¹²).

Figure 3 gives examples of sequences from the Dayhoff bank which match with parts of the 86T1 sequence. The closest match was found with a sequence just N-terminal of the third hyper-variable region of the heavy chain of the monoclonal antibody 93G7 (ref. 13). This is the most highly conserved sequence of immunoglobulin variable regions, and surrounds the second cysteine at residue 92 which forms part of the characteristic intrachain disulphide bond of the variable regions domain².

Figure 4 shows a more detailed comparison between the 86T1 sequence and those of murine immunoglobulin variable and constant regions, and demonstrates the clear immunoglobulin-like character of most of the 86T1 sequence. The similarity of the variable region of 86T1 to three different immunoglobulin variable region sequences^{13–15} is shown in Fig. 4a. The degree of similarity between 86T1 and the immunoglobulin variable regions is comparable to that between the variable regions of immunoglobulin heavy and light chains. Of the 18 invariant residues of murine κ variable regions and the 10 invariant residues of the heavy-chain variable regions, 13 and 6, respectively, are found in the 86T1 sequence. The outermost two cysteines of the 86T1 variable region are separated by a similar number of residues (68) to those separating the cysteines of immunoglobulin variable regions that form the intrachain disulphide bonds² (65 amino acids for light chains and 70 amino acids for heavy chains). The alignment in Fig. 4 also predicts that the putative leader peptide of 86T1 will be cleaved just before the asparagine (N) residue at position 20 (Fig. 2).

This similarity to immunoglobulins is also shown by the constant region of 86T1, particularly around the cysteine residue at position 164 (Fig. 4b). It is interesting to note that the region N-terminal of this cysteine is more similar to light chains and the C-terminal region is more similar to heavy chains. The similarity of the 86T1 sequence around the last cysteine residue (position 260) to both λ and κ light chains may be important as in light chains this is the cysteine that forms an interchain disulphide bond with the heavy chain. The similarity in sequence between 86T1 and immunoglobulins is so pronounced that it might at least in part explain the cross-reactivity of B-cell anti-idiotypic antisera with T cells^{16–18}. In contrast to this amino acid sequence similarity, the nucleotide sequence of 86T1 has diverged sufficiently from those of immunoglobulin genes to explain the negative results of many investigators who have used immunoglobulin gene probes to search for the genes encoding the T-cell receptor^{19–22}.

Joining region-like elements

As indicated in Fig. 2, 16 amino acids are shared between 86T1 and 86T5 but not with the other two clones that we have sequenced, 86T3 and TM86. As this falls precisely into the region occupied by joining (*J*) region elements in immunoglobulins (between the variable and constant regions) we compared this region in 86T1 with the equivalent regions of 86T3 and TM86, as well as with the consensus sequences from each of the three different immunoglobulin *J* region elements² (Fig. 5). As shown in Fig. 5, the putative *J* regions of 86T1 and TM86 both have very similar sequences to those of all the immunoglobulin *J* regions, with no need for gaps to be introduced. As with the variable region similarities, the T-cell *J*

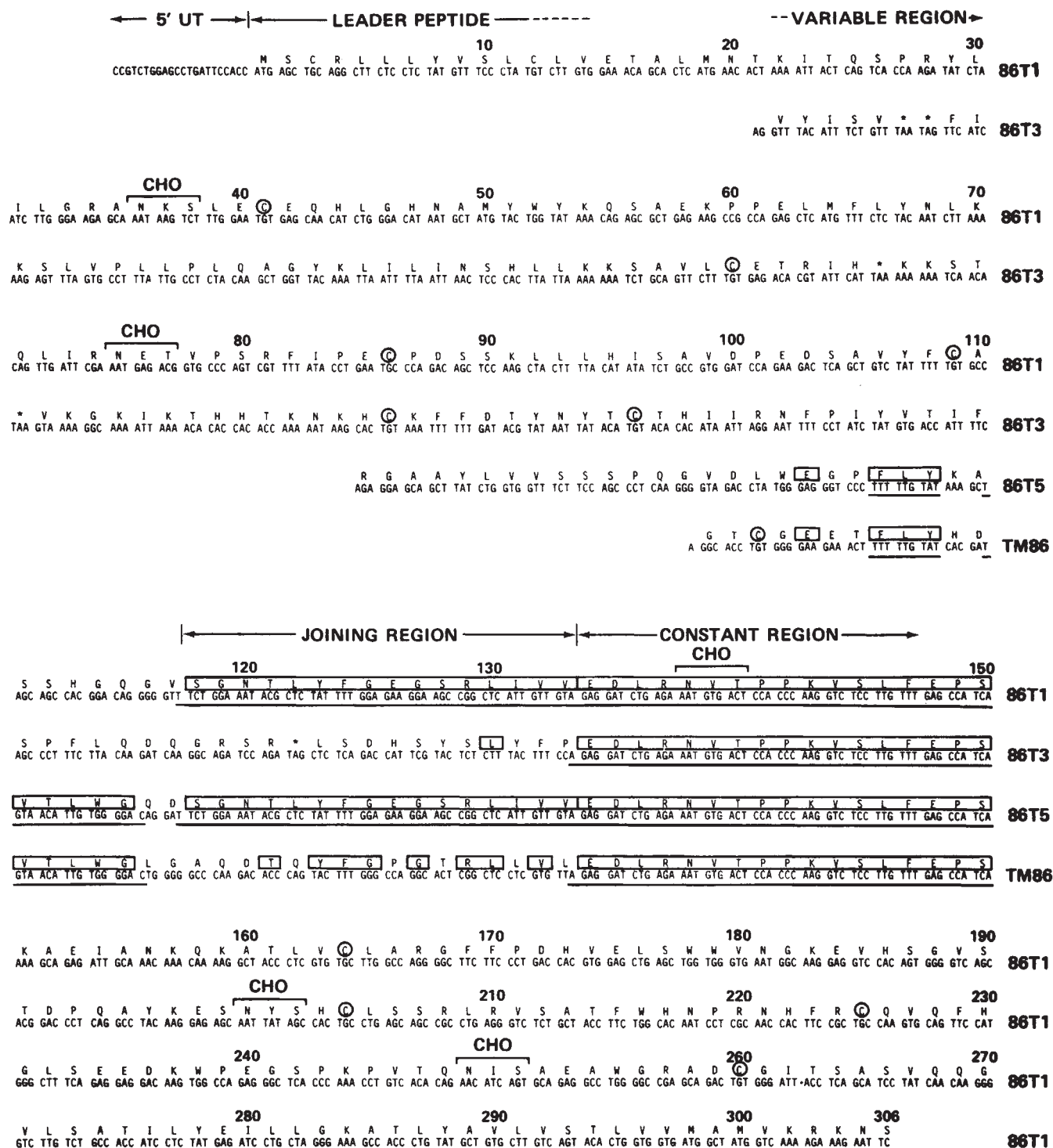


Fig. 2 Sequence of TM86 and related cDNA clones. This figure shows the complete nucleotide sequence of 86T1 and partial sequences of the other cDNA clones, indicating the 5' untranslated region (UT), the putative leader polypeptide, and the variable, joining and constant regions. Amino acid similarities are boxed, and nucleotide sequence similarities are underlined. The numbering follows the predicted amino acid sequence of 86T1. Cysteine residues are circled and possible carbohydrate (CHO) attachment sites are indicated (following the formula: N-X-S or N-X-T)³¹.

regions show an approximately equal degree of similarity to each of the different immunoglobulin *J* region types, similar to the relationship between the heavy-chain and light-chain *J* regions, but not as pronounced as that between light-chain *J* regions. In terms of size (16 amino acids) they are more closely related to heavy-chain *J* regions (17 amino acids) than light-chain *J* regions (13 amino acids) but since the putative receptor

chains are approximately equal in size²³⁻²⁵, the same constraints may not apply.

In addition to the *J* elements, the adjacent 5' regions of 86T5 and TM86 between residues 103 and 115 (Fig. 2) are very similar. In particular, the 17-nucleotide and 9-nucleotide identities between these two cDNA clones suggest the existence of other possible 'mini-gene'²⁶ elements possibly analogous to the

Score = 9.4 standard deviations above mean

100 110 120
 LLLHISAVDPEDSAVYFCASSHGQVSGNTLY
 100 110 120
 AYHQLRSLTSEDSAVYFCARSHYGGSYDFDY

86T1

V region of immunoglobulin heavy chain of 93G7

Score = 6.5

170 180 190 200
 LVCLARGFFPDHVELSWVNGKEVHSCVSTDP-QAY-KESNY
 140 150 160 170
 LICQATCFSPRQIQVSHLRECKQVSGVTDDQVQAEAKESCP

86T1

C region of human immunoglobulin μ heavy chain

Score = 5.0

140 150 160 170
 LRNVTPPKVSLFEPKAEI-ANKQKATLVCLARGFFPDHVE
 0 10 20 30
 QPKAAPSVTLPFPSSSEELQAN--KATLVCLISDFYFGAVT

86T1

C region of human immunoglobulin λ light chain

Score = 5.0

160 170
 KAEIANKQKATLVCLARGFFPDHVELSW
 240 250 260
 SSQLALNQLVTLTCLARGFSPKDVLRML

86T1

C region of human immunoglobulin α 1 heavy chain

Score = 5.0

150 160 170 180 190
 FEPSSKAEIANKQKATLVCLARGFFPDHVELSWVNGKEVHSCVSTDP
 100 110 120 130
 VTVFSPVTLGQPNLTICLVNIFPPVNNITWLSNGHSTEGVSETS

86T1

Human HLA-DC1 α chain

Fig. 3 Search of the Dayhoff sequence data bank for sequences closely related to that of 86T1 using the program of Wilbur and Lippman¹¹. Representative similarities are shown as they appear from the search with the matches indicated (the computer program focuses on short stretches of homology and thus all of the possible matches are not shown). Gaps are represented as dashes (-).

D region of heavy-chain immunoglobulins^{6,27,28}. An alternative explanation would be that these similarities represent highly conserved sequences of related variable-region genes.

Structural predictions

A number of groups have devised formulae to predict from amino acid sequences which portions of a molecule are embedded in membranes and which are exposed. These predictions are based on weighting formulae derived partly from measurements of solubility in organic and aqueous solvents and partly from empirical considerations. One such formula is that of Kyte and Doolittle²⁹ which is particularly suited for cell-surface molecules as most of the now considerable literature on the structure of such molecules has been taken into account. This 'hydropathicity' plot is shown in Fig. 6 and indicates a number of important features: first, that the 86T1 protein has the alternating hydrophobic-hydrophilic stretches characteristic of globular proteins²⁹; second, that the predicted leader polypeptide occurs in a very adequate hydrophobic environment; and most importantly, it predicts a possible transmembrane region spanning the end of the 86T1 sequence, followed by a string of positive charges (Lys-Arg-Lys) which are characteristic of the cytoplasmic portion of a number of lymphocyte cell-surface markers². The major difficulty with this prediction is the lysine residue (K) at position 284, in the middle of the putative transmembrane region, which is not characteristic, but may be masked by a salt bridge to another protein (possibly the other chain of the heterodimer).

Discussion

For the following reasons the data presented in this and the accompanying paper¹ indicate the considerable likelihood that the mRNAs related to the TM86 cDNA clone encode one chain of the T-cell antigen receptor.

Comparison with variable regions

a

20	30	40	50	60	
NTKIQSPRYL--ILGRANK-SLECEQHLGH-----NAMYWKQSAEKPPPELMF	86T1				
EVQLQSSGAEL-VRAGSSVK--MSC-KASG--YTFTSYGINWVKQRPQGGLLEWI-	V _H				
DIVMTQSPSSLSVSAG--ERVMTSCSSQSLNSGNQKNFLAWYQKPGQPPKLL-	V _K				
QAVVTQE-SALTTSPG--ETVTLTCSRSTG--AVTTSNYANWVQKPDHLFTGL-	V _{λ1}				
70	80	90	100	110	
LYNLKQLIR--NETVPSRFIPECPDSSKLLHISA-VDPEDSAVYFCASSHGQGV	86T1				
GYINPGNGYINNEKFKGKTTLTVDKSSSTAYMQLRSLTSEDSAVYFCARSHYGG	V _H				
IYGAET--R--ESGVPDRF-TGSSTGDTFTLTIS--VQAEELAVYVCNDHSHYPL	V _K				
IGGTNN--R--APGVPAF--SGSLIGNKAALTITG-AQTEDEATYFCALWYSNH	V _{λ1}				

Comparison with constant regions

b

140	150	160	170	180	
EDLRNVTPPKVSLFEPKAEIANKQKATLVCLARG-FPPDHVELSWVNGK-EVHS	86T1				
E---SQSFPNVFLVSCESPLSDKNLVANGCLARD-FLPSTISFTWYQNTTEVIQ	C _{μ1}				
P-VFIFPPSSSEELKEN-KATLVGVANK-FRPDDITVTWKVDGS-ERQN	C _K				
QPKSSPSVTLFPPSSSEELTEN-KATLVCTITD-FYPGVVTVQKVDGTPVTQ-	C _{λ1}				
E-----APQATVF-PKSPVLLG-QPNTLICFVDNITFPVINIT-WLRNSKSVTD-	A α 2				
190	200	210	220	230	240
GVSTID-PQAY-KEENYSCLSSRLVRSATFWHNPFRHRCQVQFHGLSEEDKWPEG	86T1				
GIRTF-PTLRTGG-KYLAT-SQVLLSPKSILEGSDYLVCKIHYGGKNRDLHVP-	C _{μ1}				
GVLSWTDQDSKDSYSM--SSTLTLDKDEYER-HNSYTCFAHKTSTSPIVKSFN-	C _K				
GMEITTEPS--KQSNKYMASSYLTLTAWERS-HSYSSCQVTHEGHSVEKSLSS-	C _{λ1}				
GVYETSFL--VHRDHSFHKLSYLTIFPSDDDIYD---CKVEHWGL-EEPPVLKH	A α 2				
250	260				
SPKPVVTQNISAEAWGRADCD	86T1				
-P	C _{μ1}				
-----RNEC	C _K				
-----RADCS	C _{λ1}				
W	A α 2				

Fig. 4 Similarities of the 86T1 amino acid sequence to variable (a) and constant (b) regions of murine immunoglobulins and to the second domain of the murine Ia-A₂^d (ref. 32) (the murine equivalent of the human HLA chain DC _{α}). Similarities are boxed with gaps represented as dashes (-). a Shows a comparison of the variable-region immunoglobulin sequences with the 5' region of 86T1; b shows a comparison of the 3' region of 86T1 with immunoglobulin constant-region sequences and a region of the sequences of A₂^d. The 9367/CL sequence also includes part of the D (diversity) region. The V_K is from MOPC 603¹⁴ and the V _{λ} sequence from MOPC 104e¹⁵.

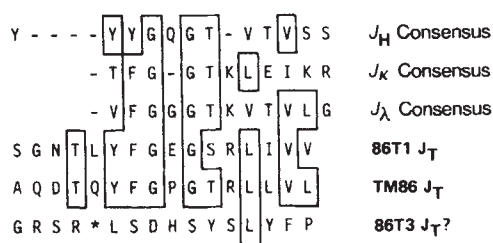


Fig. 5 Joining region sequence similarities between immunoglobulin consensus J sequences² and the apparent T-cell J regions. Similarities are boxed and dashes (-) indicate a non-conserved residue between the J region elements of a particular immunoglobulin.

The first reason is the consistent rearrangements seen in Southern blot analyses of T-cell hybridomas and lymphomas, and the fact that these appear to vary in T cells of different specificities¹. Second, the analysis presented here shows the very close similarity of the amino acid sequences predicted by our cDNA sequences to immunoglobulin variable, constant and joining regions (Figs 3-5). This similarity is much closer than that of the postulated 'super-family' of immunoglobulin-like molecules which includes a wide variety of very distantly related cell-surface glycoproteins³⁰, as judged by the outcome of the computer search (Fig. 3) and the overall similarities presented in Figs 4 and 5. Third, the finding of sequence differences and similarities between the set of cDNA clones illustrated in Fig. 2 and that these can be divided into 5' variable, 3' constant and intermediate joining regions of approximately the same size as those of immunoglobulins.

Given these considerations, we conclude that the locus described here represents a type of immunoglobulin gene specifically rearranged and expressed in at least some subsets of T lymphocytes, and that it must almost certainly play a part in antigen recognition by T cells. Supporting this later contention is recent work which indicates that antisera raised against synthetic peptide fragments of 86T1 can significantly inhibit the antigen-dependent release of interleukin 2 by T helper hybridomas (J. Rothbard, S.M.H., R.H. Schwartz and M.M.D., manuscript in preparation).

On the basis of the data that we have presented we suggest that the structure of the 86T1 gene is that shown in Fig. 7, with a 19-amino acid leader polypeptide, a 98-amino acid variable region, a 16-amino acid J region and a single globular constant-region domain followed by transmembrane and cytoplasmic portions. By analogy to immunoglobulin, the two outermost cysteines in each globular domain would be linked (as shown in the figure) and the last cysteine at position 260 would be bound to the other chain of the receptor heterodimer. It is not clear why there is an extra cysteine within each domain, but it should be noted that rabbit light-chain immunoglobulins also have an additional extra cysteine within each domain (and at the same position in the constant region); this may confer additional stability on the native molecule, perhaps through inter-chain bonding. The protein predicted from the 86T1 nucleotide sequence has a molecular weight of 34,000 (34K), and the processed molecule would be 32K, plus whatever additional coding sequence remains in the full-sized mRNA. Given the many glycosylation sites this is thus far compatible in size with either of the two 40-48K putative T-cell receptor polypeptide chains seen in the mouse^{24,25}.

Another interesting finding is the relative lack of immunoglobulin variable region homology found in the 86T5 and TM86 fragments shown in Fig. 2. 86T5, in particular, has no cysteine

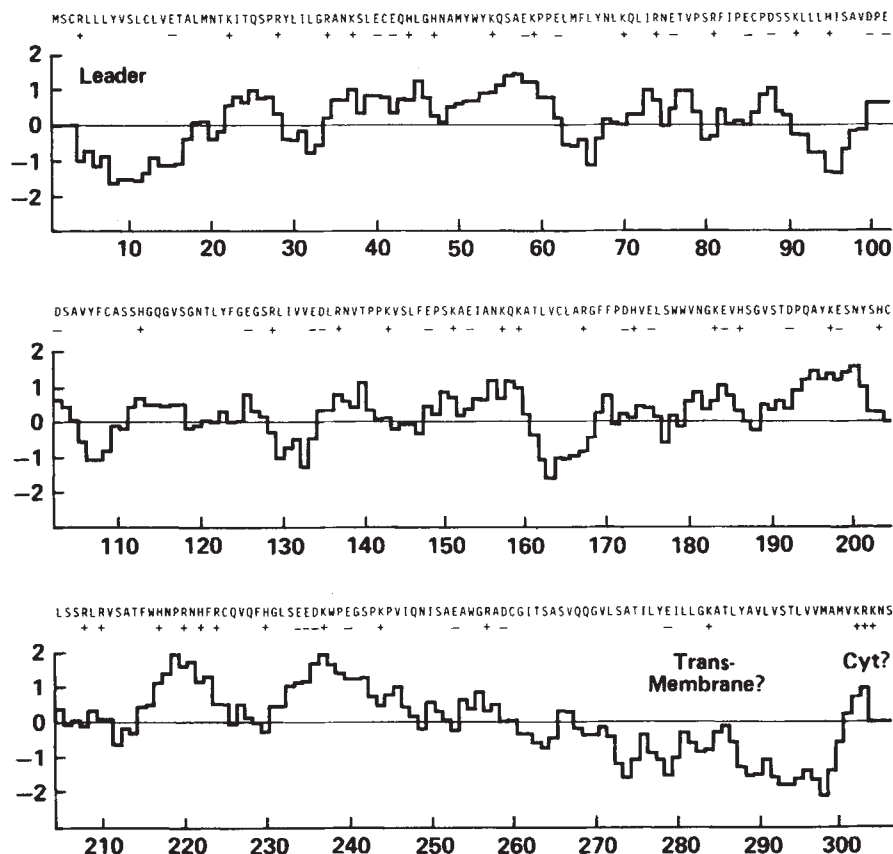


Fig. 6 Kyte-Doolittle 'hydropathicity' plot. The weighting system of Kyte and Doolittle²⁹ was used to predict membrane-embedded and hydrophilic stretches in the sequence of 86T1: negative values indicate the former and positive values the latter (see text). The numbers 1 and 2 in the figure represent the numbers 10 and 20 used in ref. 29.

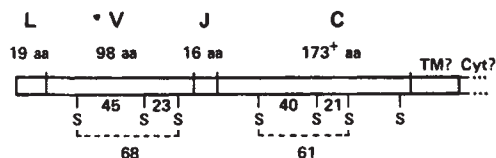


Fig. 7 Suggested structure of the 86T1 gene with leader (L), variable (V), joining (J) and constant (C) regions indicated together with the length in amino acids (aa). Also illustrated are the positions of sulphhydryl side chains of the cysteine residues (S) and possible intrachain bonding, based on the immunoglobulin analogy. The transmembrane (TM) and cytoplasmic (Cyt) portions of the molecules, based on the analysis in Fig. 6, are also shown.

residue even remotely near the one at position 109 of 86T1, which is so highly conserved in all immunoglobulin variable regions. TM86 has a cysteine nearby at position 101, but appears to lack some of the surrounding conserved sequences. This may

be the result of either a large number of non-functional transcripts (particularly in the thymus) or a wide variation in the structural requirements for a binding site.

We thank W. E. Paul for encouragement and support; C. Benoist for providing the thymocyte cDNA library; and D. Lipman for assistance in searching the Dayhoff data bank. J. Rothbards' assistance in the protein analysis was invaluable. We also thank R. Schellar, J. Rothbard and H. McDevitt for critical reading of the manuscript. D.C. is a fellow of the Leukemia Society of America. This work was financed, in part, by grants to M.M.D. from the NIH (1R01 AI20320, 1P0L AI19512).

Note added in proof: Recent sequence analysis (Y. Chien, J.K. and M.M.D., work in progress) of a cDNA clone which extends further at the 3' end than 86T1 indicates the presence of a stop codon immediately after the equivalent of residue 306 in Fig. 2. Therefore, the predicted amino acid sequence of 86T1 is most likely to be of the complete molecule.

Received 25 January; accepted 31 January 1984.

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LETTERS TO NATURE

Pioneer 10 search for gravitational waves—no evidence for coherent radiation from Geminga

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It has been reported¹ that an observed 160-min solar oscillation may be caused by gravitational radiation from the intense γ -ray source Geminga, a source whose period according to the report is exactly one cycle per year different from the solar oscillation period. This remarkable coincidence has motivated us to look for a sinusoidal component having a period near 160 min in Doppler data from the 1981 gravitational wave search with Pioneer 10. We find no such component in our most quiet data. Thus, the amplitude of a possible sinusoidal variation is no more than 3.3×10^{-14} in fractional Doppler frequency, which for the direction of Geminga translates into an upper bound of 2×10^{-14} for the polarization component of gravitational spatial strain potentially observable. This places rather stringent limits on the amount of gravitational radiation available to excite the 160-min normal mode of the Sun.

We have examined Doppler data taken by the NASA/JPL Deep Space Network with Pioneer 10 over a 3-week period from 14 November to 8 December 1981 during solar opposition; at such times low-frequency phase fluctuation caused by scattering by free electrons in the interplanetary plasma is at a minimum. The data represent part of the record from gravitational wave searches with both Pioneer 10 and Pioneer 11 during their extended Heliospheric Mission. Data are also available

from the 1982 opposition of Pioneer 10, as well as from two oppositions of Pioneer 11 for that matter, but these data have not been reduced to a point where they are useful for a search for the specific gravitational wave signal of interest here. The standard deviation of the Doppler noise from the best 1981 Pioneer 10 data is $\sim 2 \times 10^{-13}$ in fractional frequency ($\Delta\nu/\nu$) over a bandwidth of 4×10^{-5} to $\sim 2 \times 10^{-3}$ Hz. The low-frequency cutoff is set by the round-trip light-time (or 'storage' time) of the microwave link while the system noise limits the high-frequency region^{2–4}.

The long light time l to Pioneer 10, now beyond the orbit of Neptune, is a definite advantage in searching for long-period coherent gravitational waves. During the 1981 opposition $l \sim 12,490$ s and the ratio $4\pi l/P \sim 16.4$ rad, where P is the period of the hypothesized gravitational wave. This ratio enters in a fairly complicated way with the cosine of the separation angle on the sky between Pioneer 10 and Geminga, $\mu = \cos\theta$, in determining the relation between the strain amplitude h of the gravitational wave and the amplitude of the fractional Doppler response $\Delta\nu/\nu$ at the same frequency. The right ascension and declination of Pioneer 10 on 28 November 1981 was 4 h 4.05 min and 24.097° respectively, while Geminga has a position of 6 h 31 min and $17^\circ 48'$, both positions given with respect to the mean equator and equinox of 1950.0. This yields $\mu = 0.82$. From the 'three-pulse' response of the Doppler system⁵, we conclude by summing the three phasors of the coherent Geminga signal that the observable polarization component of strain amplitude of a gravitational wave at Earth is amplified by a factor of 1.63 in the Doppler data.

Two approaches were used to search for a periodic signal from Geminga in the reduced Doppler record. In the first approach, three selected long passes of Doppler data of ~ 12 h each were transformed into the frequency domain by a fast Fourier transform operating on 512 data, sampled at 100-s intervals. The three spectra were averaged with equal weight given to each; the resulting spectrum is shown in Fig. 1. No

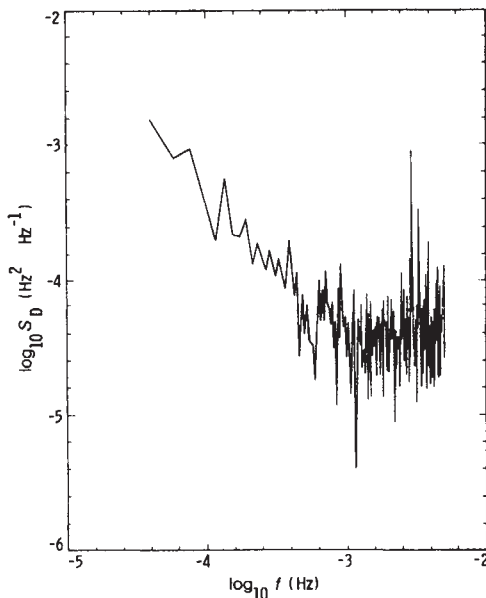


Fig. 1 Average power spectrum, S_D , of three Pioneer 10 Doppler records taken near the 1981 solar opposition. Each S-band record is 51,200 seconds long. Low frequency ($f \leq 10^{-3}$ Hz) power is dominated by interplanetary plasma scintillation noise, while higher frequency power is the result of thermal noise in the microwave receiver. A sinusoidal gravitational wave at the Geminga period would be evidenced by a spectral line near $f = 1.04 \times 10^{-4}$ Hz. The absence of any such line, in addition to least squares filtering considerations, limit the strain amplitude of an impinging gravitational wave to $|h| < 2 \times 10^{-14}$ (see text).

attempt was made to smooth the spectrum, aside from the averaging together of the individual spectra, thus estimation error is evident. Spectral lines at periods of ≤ 400 s can be explained by an aliasing of the spacecraft spin period of ~ 12.98 s and by procedures of the data reduction. Although these lines could be suppressed by a filtering out of the spin and by modifying the data reduction procedures, this is not necessary for discussing gravitational waves in the region of the spectrum where the power density is increasing with decreasing frequency.

Although there may be lines in the low-frequency end of the spectrum, at least at the Geminga frequencies corresponding to 160 min and 80 min the spectrum is depressed. At the solar oscillation frequency the Fourier power density is no more than 3.2×10^{-4} Hz² Hz⁻¹. The corresponding limiting amplitude of fractional frequency is found by multiplying the power by $4\Delta f = 7.8 \times 10^{-5}$ Hz, where Δf is the resolution bandwidth of the Fourier transform, taking the square root, and dividing by the S-band carrier frequency of Pioneer ($\sim 2.3 \times 10^9$ Hz), thus $|\Delta\nu/\nu| < 7 \times 10^{-14}$.

The second approach to finding a coherent signal in the Doppler record was to assume a Geminga period of 159.96 min and to use all the low-noise data from the 1981 opportunity to determine the amplitude and phase of the signal by least squares. In this way an accumulated observing time of 3.74 days out of the total 3-week opportunity was obtained. The overall standard deviation on the low-noise data was 4.5×10^{-13} in fractional frequency. For a signal of the form, $A \cos(\omega t) + B \sin(\omega t)$, with t measured from the arbitrary epoch of 26 November 1981 01 h 25.7 min, the least-squares approach yielded

$$A = (-1.47 \pm 1.16) \times 10^{-14} \quad (1)$$

$$B = (-1.50 \pm 1.16) \times 10^{-14} \quad (2)$$

with a corresponding amplitude of $|\Delta\nu/\nu| = (2.1 \pm 1.2) \times 10^{-14}$. The quoted uncertainties are formal in the sense that the assumed standard deviation of the Doppler data is set equal to the r.m.s. residual after the fit. Hence the formal 1σ upper bound on the amplitude is 3.3×10^{-14} . On the other hand, the preceding spectral limit on the amplitude was derived from an

integrated record of 512 points at a 100-s sample rate over 0.59 days. At best this limit could be improved by a factor of $\sqrt{3.74/0.59}$ by optimally using all the low-noise data, or consequently from a level of 7×10^{-14} to 2.8×10^{-14} . To obtain a more realistic estimate of the actual limit, and to allow for coloured noise, we performed least-squares estimates for the hypothesized Geminga signal from 100 realizations of simulated data records containing no signal but with noise corresponding to a flicker ($1/f$) turbulence spectrum similar to that of Fig. 1. The results showed that the least-squares solution from the real data was in fact typical and also that it was consistent with the hypothesis of no signal at the Geminga frequency. Further, the 1σ upper bound on $\Delta\nu/\nu$ of 3.3×10^{-14} from the 3.74 days of low-noise data was indeed about what we would expect, given our noise level and integration time. Hence, by adopting the least squares upper bound, and then using the amplification factor mentioned above, we have derived a limit $h < 2 \times 10^{-14}$ for the possible amplitude of a sinusoidal gravitational wave from Geminga.

We also looked at the previously published spectra from Voyager 1 (ref. 6). Fortunately Voyager was also favourably positioned for the detection of sinusoidal variations at a 160-min period and it did have one advantage in that unusually low phase variations in the neighbourhood of 10^{-4} Hz were recorded on 12 March 1980, thus a bound comparable to Pioneer 10 could be set for that one day.

If the 160-min solar oscillation mode is continuously excited by gravitational radiation from Geminga, it is reasonable to assume that it closely approximates a damped linear oscillator, at least if nonlinear amplification mechanisms are neglected, thus the expected steady-state surface oscillation will have an amplitude given by

$$\Delta d \approx h R_\odot Q \quad (3)$$

where $R_\odot$ is the solar radius and Q is a quality factor for the particular 160-min mode of interest. The corresponding surface velocity would be

$$v \approx \frac{2\pi}{P} h R_\odot Q \quad (4)$$

and for the maximum value of h allowed by the Doppler data, $v \approx 9 \times 10^{-7} Q$ cm s⁻¹.

The observed velocity as given by Scherrer and Wilcox⁷ is 17 cm s⁻¹, which would require a Q of the order of 2×10^7 and a correspondingly long stable lifetime for the source, if, as suggested by others, it is responsible for the solar oscillation at the observed amplitude and resonant frequency. If one wishes to invoke nonlinear amplification mechanisms in outer regions of the Sun, then at the same time a smaller value for R is needed in keeping with the dimensions of a resonant core. Thus any lowering of Q , assuming smaller oscillations in the core, is offset to some degree by the smaller value of R .

The most probable source for a sinusoidal gravitational wave of 160-min period is a close binary system⁸⁻¹¹ but a wave produced by a binary at the maximum Geminga distance of 100 pc¹² would require supermassive components in order to have an amplitude equal to the limiting strain amplitude of 2×10^{-14} from our analysis. The minimum total mass M of the binary would be at least $4 \times 10^4 M_\odot$ with a corresponding gravitational luminosity L of 2×10^{45} erg s⁻¹, and remaining lifetime Δt of ≤ 80 yr in contradiction to the long lifetime needed for a high Q resonance. For comparison, the γ -ray luminosity, which presumably dominates the electromagnetic spectrum, is of the order of 10^{12} times smaller. For a given period these gravitational properties depend on the product of wave amplitude h and distance r as $M \propto (hr)^{3/5}$, $L \propto (hr)^2$, $\Delta t \propto 1/(hr)$. Even if the source is at a distance of only 10 pc and if the strain amplitude is a factor of 1,000 smaller than the Pioneer 10 limit, the mass of the system would be $150 M_\odot$ and $L \approx 10^{37}$ erg s⁻¹, still a very luminous source of gravitational radiation.

Finally, we must consider the possibility that Geminga is a binary seen edge-on, and hence that it is a source of plane-

polarized gravitational waves. If in addition the plane of polarization is inclined 45° to the ecliptic, then neither Pioneer 10 nor Voyager 1 would be particularly sensitive to its gravitational wave. Yet barring this double coincidence it seems unlikely that any quadrupole radiation available to stimulate the Sun is more than a factor of $\sqrt{2}$ larger than the 2×10^{-14} limit set by Pioneer 10. Eventually other spacecraft (Pioneer 11, Voyager 1 and the International Solar-Polar Mission) that are not in the plane of the ecliptic will be distant enough also to respond to coherent radiation from Geminga at different alignment angles.

We thank the NASA/JPL Deep Space Network for their cooperation in the acquisition of data, and J. M. Rotenberg and G. B. Trager for their assistance in the data reduction. This work was supported by the Pioneer Project Office at NASA/Ames Research Center under Letter of Agreement ARC/PP017 for J.D.A. and E.K.L., and by the NASA Office of Astronomy/Relativity for J.W.A., F.B.E., R.W.H. and H.D.W. The work was performed at the Jet Propulsion Laboratory under contract with NASA.

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Geminga and the 160-min solar oscillation

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The idea that solar oscillations might have been stimulated by gravitational radiation from a nearby binary system^{1,2} has recently been explored by Delache and co-workers^{3,4}. They have announced that the γ -ray source CG195+4, known as Geminga, varies in intensity with a period of 159.96 min (ref. 3), and therefore has a frequency just $(1 \text{ yr})^{-1}$ greater than the 160.01-min solar oscillation^{5–7}. From this coincidence they have inferred a gravitational connection between the two oscillations. We show here that if the generally accepted ideas of gravitational radiation are correct, the 160-min solar oscillation could not have been driven to its observed amplitude by any binary system of stellar mass. Only if there were a sustained resonance between the incident radiation and a solar mode of oscillation could there be any chance of an observable response. However, gravitational radiation causes the binary system to spin up, prohibiting it from remaining in resonance with a 160-min mode for long enough to have a perceptible effect. It is just possible that 5-min oscillations in the Sun could be excited to an observable amplitude by a binary system having an orbital period of ~ 10 min at 1,000 AU.

The response of the Earth to non-resonant gravitational radiation is discussed by Dyson⁸. Some aspects of the analysis for the Sun are similar. Within the framework of general relativity⁹, the metric tensor in the wave zone far from a binary system is distorted from its galilean value by a small quantity with spatial components h_{ik} . In a galilean coordinate frame S whose origin

coincides with the centre of mass of the Sun, material at the spatial coordinate x_k experiences an acceleration $f_i = h_{ik}x_k$. We are assuming the coordinates to be cartesian, so we need not distinguish between covariant and contravariant components of a tensor. Thus, the displacement 3-vector ξ_i of small-amplitude solar oscillations driven by this force satisfies the linearized equation of motion:

$$\rho \frac{\partial^2 \xi_i}{\partial t^2} - \mathcal{L}_{ik} \xi_k = \rho f_i \quad (1)$$

where ρ is the equilibrium matter density of the Sun, t is time and $\mathcal{L}_{ik}$ is the usual spatial differential operator describing linearized solar oscillations^{10,11}. We begin by disregarding the evolution of the equilibrium solar structure.

For the oscillations discussed here, the nonadiabatic contribution to $\mathcal{L}_{ik}$ is small, so we approximate the spatial structure of ξ_i by its adiabatic counterpart. This is adequately represented by the eigenfunctions of a nonrotating solar model. Then we know that these spatial eigenfunctions $\Xi_i^{n\ell m}$ of free oscillation form a complete set^{12,13}. Hence we may represent ξ_i as a linear combination $X^{n\ell m}(t) \Xi_i^{n\ell m}(x_k)$, which is summed over all orders n , degrees ℓ , and azimuthal orders m of the normal modes. We then project equation (1) onto any chosen eigenfunction, Ξ_i (from now on we omit the superscripts), by taking the scalar product of the equation with the adjoint Ξ_i^* of that eigenfunction and integrating over the volume of the star. We treat the nonadiabatic terms as a small perturbation, and ignore the possibility of accidental degeneracies, and so obtain for the time dependence of the chosen mode:

$$\frac{d^3 X}{dt^3} + \omega_0^2 \frac{dX}{dt} - Q^{-1} \omega_0^3 X = \langle \rho \Xi_k^* \Xi_k \rangle^{-1} \frac{d}{dt} \langle \rho \Xi_i^* f_i \rangle \quad (2)$$

where the angular brackets represent the integration, ω_0 is the adiabatic eigenfrequency and Q is the quality factor of the oscillation, which at first we presume to be positive.

We accept the standard discussions of gravitational radiation from a binary system^{9,14}. For simplicity, we assume the binary orbit to be circular with frequency $\frac{1}{2}\omega$; in that case the perturbation h_{ik} varies sinusoidally in time with frequency ω . A γ -ray source such as Geminga is probably a compact object such as a neutron star or black hole. The γ rays are then due either to accretion of matter transferred from its more normal stellar companion, or to pulsar action. If the Earth lies close to the orbital plane, simple eclipses by the stellar companion occur at frequency $\frac{1}{2}\omega$, only half that of the gravitational radiation. This discrepancy could be removed by invoking either two γ -ray emission regions⁴ or a disk-shaped beam that rotates at the binary frequency $\frac{1}{2}\omega$. For the present we assume that the line-of-sight to the Earth is in the orbital plane, and consider gravitational radiation only in that plane.

The perturbed metric at the Sun is most easily described in the frame S with respect to a cartesian coordinate system x_i such that $(0, x_2, x_3)$, say, lies in the orbital plane of the binary system and the binary system lies on the x_3 axis. Then

$$h_{ik} = \Lambda \begin{pmatrix} \cos \int \omega dt & 2 \sin \int \omega dt & 0 \\ 2 \sin \int \omega dt & -\cos \int \omega dt & 0 \\ 0 & 0 & 0 \end{pmatrix} \quad (3)$$

where

$$\Lambda = \frac{4 \mu (GM)^{5/3} (\frac{1}{2}\omega)^{8/3}}{(1 + \mu)^2 c^4 d} \quad (4)$$

μ is the mass ratio and M the total mass of the source, d is the distance to the source and G is the gravitational constant. It is then a straightforward matter to compute the forcing term on the right-hand side of equation (2).

Only non-radial modes of even degree ℓ having $m \leq 2$ can be excited. Of these, the forcing of the quadrupole modes is normally the greatest. If coincidence of the sidereal frequencies of the Sun and the source were important one could deduce from the $(1 \text{ yr})^{-1}$ sidereal-synodic frequency difference reported

Table 1 Magnitudes of the geometrical coefficients β for a selection of modes with $m=2$ of solar model A of ref. 32

$\ell=2$			$\ell=4$			$\ell=6$		
Mode	Period (min)	$ \beta $	Mode	Period (min)	$ \beta $	Mode	Period (min)	$ \beta $
g_9	157	5.7×10^{-3}	g_{17}	160	4.3×10^{-4}	g_{24}	158	5.3×10^{-5}
g_5	102	2.2×10^{-2}	g_{10}	104	4.5×10^{-3}	g_{15}	107	3.3×10^{-4}
g_3	77.1	5.3×10^{-2}	g_7	81.8	1.9×10^{-2}	g_{10}	80.6	1.5×10^{-3}
g_1	57.3	0.79	g_1	46.9	0.12	g_1	43.5	1.3×10^{-3}
f	48.2	0.50	f	41.5	7.1×10^{-2}	f	40.0	2.3×10^{-3}
κ_1	43.4	0.70	κ_1	37.6	1.76	κ_1	33.8	1.23
κ_{10}	10.1	26.7	κ_9	10.3	41.4	κ_8	10.7	25.8
κ_{22}	5.1	38.8	κ_{21}	5.1	86.0	κ_{10}	5.2	78.1
κ_{30}	3.8	26.4	κ_{29}	3.8	64.3	κ_{28}	3.8	66.4

These coefficients relate to the surface displacement amplitude, and so are greater for high-order κ -modes. The cross-section for gravitational energy absorption scales roughly as $[n^2 + \ell(\ell+1)]^{-1} \beta^2$ for low-degree g -modes, and decreases with n somewhat faster for κ -modes, and so is greatest for modes of low order. No significance should be attached to the fact that only for $\ell=4$ is there a g -mode with a period of exactly 160 min; for any given ℓ it requires only a slight modification to the equilibrium structure to yield a mode with a 160-min period. Values of $|\beta|$ for other azimuthal orders can easily be deduced from equation (8), since the eigenfunctions (Ξ , H) are essentially independent of m .

in refs 3 and 4 that the mode cannot be axisymmetric. Moreover, modes having odd $\ell+m$ cannot be detected by the instruments that have been used to observe the 160-min solar oscillation¹⁵. Therefore, we restrict attention to modes with $m=2$ propagating around the rotation axis of the Sun in the same direction as the orbital velocity of the Earth.

Because the binary system radiates gravitational waves, it loses energy and so spins up. The spin-up rate is very much less than ω , and so can be computed by perturbing about a precise balance between newtonian gravitational and centrifugal forces. The result is¹⁴

$$\varepsilon = \omega^{-2} \frac{d\omega}{dt} = \frac{24\Lambda d}{5c\omega} \quad (5)$$

which is small. For two objects of $1 M_\odot$ orbiting each other in a period of 320 min, for example, $\varepsilon = 3 \times 10^{-14}$. Thus, the Sun is subjected to oscillatory forcing with a slowly varying frequency. In terms of the usual vertical and horizontal displacement amplitude-eigenfunctions (Ξ , H) of stellar pulsation theory^{10,11,16} (expressed in spherical polar coordinates r , θ , ϕ about the rotation axis), that forcing may be written:

$$\langle \rho \Xi_k^* \Xi_k \rangle^{-1} \frac{d}{dt} \langle \rho \Xi_i^* f_i \rangle = \alpha \Lambda \operatorname{Re} \left(i\omega \exp i \int \omega dt \right) \quad (6)$$

with

$$\alpha = (\cos^2 2\Theta + 4 \sin^2 2\Theta)^{1/2} \beta \quad (7)$$

and

$$\beta = \begin{cases} -\frac{1}{\sqrt{15}} \frac{\langle \rho(\Xi^* - \frac{3}{4}H^*) \rangle}{\langle \rho[\Xi^*\Xi + \ell(\ell+1)H^*H] \rangle}, & \ell=2 \\ 3 \left[\frac{(2\ell+1)(\ell-|m|)!}{2(\ell+|m|)!} \right]^{\frac{1}{2}} \frac{\langle \rho H^* \rangle}{\langle \rho[\Xi^*\Xi + \ell(\ell+1)H^*H] \rangle}, & \ell \geq 4 \end{cases} \quad (8)$$

where Θ is the angle subtended by the plane of the binary orbit and the equatorial plane of the Sun. The functions Ξ , H have been normalized to yield a r.m.s. vertical displacement amplitude of $R_\odot$ at the surface ($r=R_\odot$) of the Sun. It is adequate to use the real eigenfunctions of a non-rotating solar model (which satisfy $\Xi^* = \Xi$, $H^* = H$) in expressions (8).

The amplitude of the forced oscillations may be derived from a two-time analysis¹⁷ of equation (2). A physical discussion is given by Pippard¹⁸. Since ω hardly varies over an oscillation period, we introduce a dimensionless evolutionary time variable $\tau = \varepsilon^{1/2} \omega_0(t - t_0)$, where t_0 is the time at which resonance occurs, set

$$X = \operatorname{Re} \left[A(\tau) \exp i \int \omega dt \right] \quad (9)$$

and assume $|A^{-1} dA/d\tau| = 0(1)$. Substituting expression (9) into

equation (2) and retaining only the dominant terms when $\varepsilon \ll 1$ yields

$$2\psi \frac{dA}{d\tau} + \left[\frac{d\psi}{d\tau} + (\varepsilon^{1/2} Q\psi)^{-1} - i\varepsilon^{-1/2}(1-\psi^2) \right] A = i\varepsilon^{-1/2} \omega_0^{-2} \alpha \Lambda \quad (10)$$

where $\psi = \omega_0^{-1} \omega$ is a dimensionless frequency whose evolution is determined by equation (5).

The nature of the solutions of equation (9) depends on the magnitude of Q . If $Q \lesssim \varepsilon^{-1/2}$ the natural decay time of the oscillations is less than the evolution time, even as the forcing frequency passes through resonance. The oscillation stays in quasi-equilibrium with the forcing, having amplitude

$$|A| \approx \alpha \Lambda \omega_0^{-2} [1 + Q^2 \psi^2 (1 - \psi^2)^{-1/2}]^{-1/2} Q \psi \quad (11)$$

On the other hand, when $Q \gtrsim \varepsilon^{-1/2}$ equilibrium is not achieved near maximum amplitude because the forcing frequency varies too rapidly. The amplitude rises predominantly whilst τ is within about $\pi/2$ of resonance, after which the system is left oscillating, at amplitude $|A| = A_m$, until it decays naturally on a dimensionless time scale of εQ . There is a systematically increasing difference between the phase of the forcing and that of the oscillator during this time. Dissipation has no significant role during the excitation phase, and the amplitude A_m is independent of Q . It follows from equation (10) that

$$A_m \approx \frac{1}{2} \varepsilon^{-1/2} \alpha \Lambda \omega_0^{-2} \left. \frac{L t}{T \rightarrow \infty} \right| \int_{-\tau}^{\tau} e^{i\sigma^2/2} d\sigma \quad (12)$$

$$= (\pi/2\varepsilon)^{1/2} \alpha \Lambda \omega_0^{-2}$$

This formula is asymptotically correct in the limit $\varepsilon^{1/2} Q \rightarrow \infty$, provided $1 \ll \tau \ll \varepsilon^{1/2} Q$, though it provides a good estimate even when $\varepsilon^{1/2} Q = 0(1)$. For example, A_m is within about 25% of this limit when $Q \gtrsim 3\varepsilon^{-1/2}$.

A direct computation of Q for the 160-min solar oscillation has not been performed. Nevertheless, one can extrapolate from the quasi-adiabatic computations^{16,19,20} of modes of lower order, obtaining $Q \approx 2 \times 10^9$. This may be an overestimate, because these calculations do not account for the potentially severe damping in the upper part of the convection zone. Indeed, the admittedly uncertain nonadiabatic calculations^{21,22} suggest that Q may be as low as 5×10^7 . In either case, however, the theoretical estimates suggest that the Sun responds to gravitational radiation as a truly high- Q oscillator, in the sense that $Q > \varepsilon^{-1/2}$, so it is not necessary to know the value of Q in order to calculate the maximum amplitude of the forced oscillations. For a source of given total mass M , the greatest displacement amplitude, according to equations (12), (4), (5) and (7), is

$$A_m \approx \left(\frac{5\pi}{96} \right)^{1/2} |\beta| \left(\frac{GM}{c^2} \right)^{5/6} \left(\frac{2c}{\omega} \right)^{1/6} \frac{R_\odot}{d} \quad (13)$$

and occurs when $\mu = 1$ and $\Theta = \pi/4$.

Table 1 lists the geometrical coupling factors $|\beta|$ for a variety of low-degree modes with $m = 2$. The coupling, which is defined in terms of the surface displacement amplitude and not the energy, is strongly dependent on frequency. The 160-min oscillation, which is a typical low-degree g -mode, is trapped beneath the convection zone, and so produces a relatively low response in the photosphere. Conversely, the higher frequency p -modes have a nearly uniform distribution of energy per unit radial distance, and so produce a relatively large disturbance in the low-density atmosphere. We predict that the power in a Doppler measurement of a 5-min quadrupole mode could be more than 10^{10} times greater than that of a quadrupole 160-min mode, if both were excited by binary sources of the same total mass M at the same distance d .

Even though we admit that standard solar models may not be an accurate representation of reality, we note that under no circumstances could the 160-min oscillation be a p -mode²³. The longest quadrupole p -mode period for a stable star with a solar mass and radius is ~ 52.4 min. It is most unlikely that $|\beta|$ could exceed unity for any g -mode.

According to equation (13), the photospheric velocity of the 160-min quadrupole mode with $m = 2$ should be excited to a maximum amplitude of

$$V_m \approx 3.0 \times 10^{-4} \left(\frac{M}{M_\odot} \right)^{5/6} d^{-1} \text{ m s}^{-1} \quad (14)$$

where d is measured in AU. The amplitudes of modes of higher degree are no greater. Thus to achieve the observed amplitude^{5,7} of $\sim 0.5 \text{ m s}^{-1}$ with $M = M_\odot$ would require $d \leq 6 \times 10^{-4} \text{ AU}$, which is out of the question. If, on the other hand, it were assumed that the source is situated 10 pc from the Solar System, a naive application of equation (14) would lead to a total mass $M = 3 \times 10^{11} M_\odot$. Once again this is not possible: not only would the implied binary orbit lie within its own Schwarzschild radius, but also the mass required is comparable with that of the entire Galaxy.

It follows from equation (5) that the e-folding time for the increase in ω is $\sim 3 \times 10^9 \text{ yr}$, for a 320-min binary system composed of two objects of $1 M_\odot$. This is comparable with the corresponding time, about 10^{10} yr , for the increase in g -mode eigenfrequencies resulting from the evolution of the Sun. Thus by ignoring the evolution we have overestimated the rate at which the gravitational wave frequency passes through resonance. This does not substantially affect our conclusion: even if the effective value of ϵ were reduced by a factor of 10, the distance d at which it would be necessary to envisage the binary system would need to be increased by only a factor of 3. Solar evolution theory predicts a different variation in ω_0 from that implied for ω by equation (5). Therefore an even greater accidental reduction in ϵ is quite unlikely. We note that a chance resonance at 160 min between two modes, ignored in the derivation of equation (2), would also modify our result only by a factor of order unity.

We conclude, therefore, that provided the linear adiabatic eigenfunctions of free oscillation faithfully represent the spatial

structure of the forced motion, the 160-min solar oscillation cannot have been excited by gravitational radiation.

We note also that even if g -modes were self-excited, for example by the thermonuclear energy generation^{16,19-22}, we would expect the excitation to be fairly broadband. If $|Q|$ were small enough to have caused the 160-min mode to have grown to its present amplitude in less than $3 \times 10^4 \text{ yr}$, any gravitational forcing probably would have been too weak to have played a significant part in selecting that mode. If, on the other hand, the Sun by chance had become unstable to g -modes just as Geminga was approaching resonance, and $|Q|$ were large enough for the gravitational radiation to have picked out the 160-min mode (which subsequently suppressed the others), then the time taken for the selected mode to have grown to the amplitude observed would have been so great that Geminga would now no longer have the same period as the solar mode. The possibility that different g -modes are excited sequentially by gravitational radiation and that energy accumulates by nonlinear mode interactions is also unlikely, partly because the number of suitable modes that can be strongly coupled to the forcing field is too low.

It is possible that a 160-min solar oscillation is not a normal mode; for example, it may be a solitary wave²⁴ or a subharmonic of the p -modes²⁵, both of which depend on nonlinear interactions. We estimate that in these cases too, the influence of gravitational radiation is negligible.

The situation is rather different for 5-min normal modes. In that case $V_m \approx 40 d^{-1} \min(1, \epsilon^{1/2} Q) \text{ m s}^{-1}$ for damped quadrupole modes if $M = M_\odot$ and d is measured in AU, the high- Q limit being attained if the natural decay time of the solar modes exceeds $\sim 1 \text{ yr}$. From analysing the accelerations of pulsar periods, Cowling²⁶ has set a lower limit of 770 AU on d for a $1 M_\odot$ object. This does not preclude the possibility of excitation of 5-min modes to observable amplitudes, although it would be difficult to ascertain whether it is really gravitational radiation that is responsible.

A coincidence between the periods of Geminga and a prominent solar oscillation leads one to suspect a causal link. We cannot identify any plausible mechanism. The hard γ -ray flux from Geminga seems to be too low to have any measurable influence on either the Sun or the Earth's atmosphere through which the solar oscillations are observed. Low-frequency electromagnetic waves from a binary system, or pulsar, are unlikely to propagate sufficiently far through the interstellar medium. Moreover, we cannot explain the 160-min variations in the solar IR brightness²⁷ and radio polarization²⁸, and in the geomagnetic field²⁹; the near commensurability of 160-min with the spin periods of most of the planets³⁰ and of the major asteroids³¹; and the correlation of the 160-min oscillation with the occurrence of earthquakes.³¹

We thank G. F. Bignami, A. B. Callear, P. Campbell, W. Däppen, D. W. Dewhurst, J. Farman, D. Freeman, G. W. Gibbons, D. Gubbins, A. Hewish, D. Lynden-Bell, J. E. Pringle, M. J. Rees, P. Thaddeus, B. A. Thrush, G. Vaughan and C. D. Walshaw for helpful conversations.

Received 21 November; accepted 30 December 1983.

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Resonant reception in the Solar System of gravitational waves from external sources

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Here we aim to point out the rather stringent limitations, particularly those due to frequency stability requirements, that can be placed on conceivable (for example, binary) sources of any gravitational waves that might be detected by resonant stimulation of an oscillation mode in the Solar System. This question has recently been raised by widespread informal discussions^{1,2} of the possibility that unexpectedly persistent low-frequency (160-min period) oscillations observed in the Sun³ might be due to such a mechanism⁴. We show here that this particular possibility can be excluded.

The most efficient sources of steady gravitational waves that can easily be imagined are gravitationally bound binary systems, whose radiation frequency ν , say, must necessarily be variable, at a rate $\dot{\nu}$ say, due to the effect of loss of the energy that is carried away by the gravitational radiation itself (such a variation, at just the rate predicted by Einstein's theory, has recently been observed directly for the first time in the case of the binary pulsar, PSR1913+16 by Taylor *et al.*⁵).

We shall recapitulate some basic order of magnitude inequalities that are relevant for stimulation of a receptor oscillation mode of period τ_0 say (which would be ~ 160 min in the application we have in mind) by any such slowly variable source of steady waves with characteristic amplitude h . The corresponding dimensionless resonance amplitude, α , can build up to an order of magnitude

$$\alpha \approx n h \quad (1)$$

after n oscillation periods. This number of periods is either the receiver quality Q , if the receiver saturates, or is given by the time needed for the emitter frequency to sweep through the relevant 'effective bandwidth' of the receiver which in general will be of the order of $1/n$ (which reduces to the standard value $1/Q$ in the case of saturation).

Let T be the (presumably long) lifetime characterizing the time scale of variation of the source frequency, as defined by $\dot{\nu} \approx \nu_0/T$. Then we obtain

$$n^2 \approx \min \{Q^2, T/\tau_0\} \quad (2)$$

so that the criterion for achieving saturation is $T \geq Q^2 \tau_0$. In the absence of any knowledge of Q we cannot know whether this condition is satisfied, but in either case we can be sure that the amplification factor n is subject to the upper limit

$$n^2 \leq T/\tau_0 \quad (3)$$

The time scale Δt during which the oscillation amplitude will be maintained at a value comparable with the maximum given by equation (1) is also determined by the interval during which the emitter frequency remains within the effective bandwidth if saturation is achieved, but otherwise it will simply be given by the damping time, so we obtain

$$\Delta t \approx \max \{T/Q, Q\tau_0\} \quad (4)$$

Our purpose here is to point out that one can use equation (3) to obtain decisive limitations on the nature of the

hypothetical source merely from a knowledge of the directly observable quantities α and τ_0 . Prolonged observation can provide at least a lower limit on the value of Δt , but in order to exploit this using equation (4) one would need some information about Q .

Let us now use these quite general conclusions to test the hypothesis that the source is a binary system consisting of two masses, M and $m \leq M$ say, in a circular orbit for which the appropriate period would be

$$P = 2\tau_0 \quad (5)$$

The resulting dimensionless amplitude h of the waves at a distance R from the source will have an order of magnitude given, according to Einstein's theory (see, for example, ref. 6) by

$$h \approx G^{5/3} \mu M^{2/3} / c^4 P^{2/3} R \quad (6)$$

where $\mu = mM/(m+M) < M$ is the reduced mass and G is Newton's constant. The corresponding time scale T characterizing the change of frequency due to gravitational energy loss will be given (see, for example, ref. 6) by

$$T^{-1} \approx G^{5/3} \mu M^{2/3} / P^{8/3} c^5 \quad (7)$$

which implies

$$hT \approx cP^2/R \quad (8)$$

which is independent of the assumed mass values.

If we have reason to believe that the saturation condition is satisfied, this last relation can be conveniently used in conjunction with the inequality

$$hT \geq \alpha \Delta t_0 \quad (9)$$

obtained from equations (2) and (4), where Δt_0 is an observational lower limit on Δt . This gives the upper limit

$$R/\lambda_0 \leq \tau_0/\alpha \Delta t_0 \quad (10)$$

on the distance R to the hypothetical source in terms of the observables α , τ_0 and Δt_0 , where

$$\lambda_0 = c\tau_0 \quad (11)$$

is the wavelength of the radiation.

In order for the effect of the source to be describable at all in terms of gravitational radiation rather than in terms of direct newtonian inverse-square law interaction, the receptor must be situated in the wave zone, that is, at a distance

$$R \geq \lambda_0 \quad (12)$$

which will be compatible with equation (10) only if

$$\alpha \leq \tau_0/\Delta t \quad (13)$$

This extreme upper limit on the amplitude of resonant excitation obtainable from gravitational radiation emitted by a binary source is already barely satisfied by the data for the 160-min oscillations observed in the Sun, which appear to have maintained a dimensionless magnitude $\alpha \approx 10^{-5}$ over a time scale Δt_0 of the order of 10 yr, which corresponds to $\Delta t_0/\tau_0 \approx 3.0 \times 10^4$. This means that if there were a gravitational binary source it would barely be in the wave zone (requiring a mass $M \approx 10^3 M_\odot$) at a maximum distance comparable with that of the outer planets of the Solar System, which is obviously out of the question.

If Q were greater than $\Delta t/\tau_0$, the preceding deduction would not apply but we can nevertheless obtain a generally valid upper limit on the distance R by applying equation (3) in conjunction with equation (8), which gives

$$\frac{R}{\lambda_0} \leq \frac{1}{\alpha n} \quad (14)$$

Applying this in conjunction with equation (6) and using the inequality $\mu \leq M$ we obtain [as a generally valid alternative to equation (13)] the inequality

$$\alpha^{6/5} \leq \left(\frac{GM}{Rc^2} \right) \left(\frac{\lambda}{R} \right)^{1/5} \quad (15)$$

Subject to (12) this implies an *absolute* lower mass limit

$$M \geq \alpha^{6/5} (\lambda_0 c^2 / G)$$

(which gives $M \geq 10^3 M_\odot$ for the solar oscillations in question).

Now the maximum mass M of any realistically conceivable object at a given distance R from the Solar System is subject to several observational limitations. In particular the acceleration $a \approx GM/R^2$ to which we would be subject as a result is limited by the well known fact (see, for example, Radhakrishnan⁷) that many pulsars have a frequency decay time scale, T_p , as long as 10^8 yr, whereas none is known to have a frequency acceleration. In order that the corresponding Doppler frequency variation time scale should not be shorter than this maximum value, T_p , we must have $a \leq c/T_p$. Therefore, in terms of a length scale L of the order of one light year, which is conveniently comparable with typical interstellar separation distances, we deduce that any hypothetical massive object in our neighbourhood is subject to the condition

$$\left(\frac{L}{R}\right) \left(\frac{GM}{Rc^2}\right) \leq \frac{L}{cT_p} \quad (16)$$

where $L/cT_p \approx 10^{-8}$. Furthermore, in order not to be detectable by its effect on the velocity field of nearby stars, the value of GM/L for such an object cannot be too large compared with their characteristic velocity dispersion $\bar{v}^2$, which in practice corresponds to a value of $\bar{v}$ of the order of a few hundred kilometres per second. This gives the upper limit:

$$\frac{GM}{Lc^2} \leq \frac{\bar{v}^2}{c^2} \quad (17)$$

where on a conservative estimate $\bar{v}^2/c^2 \approx 10^{-5}$, so that the maximum conceivable value of M is $\sim 10^6 M_\odot$. Applying equations (16) and (17) in conjunction with equation (15) we obtain

$$\alpha^{6/5} \left(\frac{L}{\lambda}\right)^{1/5} \leq \min \left\{ \left(\frac{L}{cT_p}\right) \left(\frac{R}{L}\right)^{4/5}, \left(\frac{\bar{v}^2}{c^2}\right) \left(\frac{L}{R}\right)^{6/5} \right\} \quad (18)$$

This inequality is optimized when

$$\left(\frac{R}{L}\right)^2 \approx \left(\frac{\bar{v}^2}{c^2}\right) \left(\frac{cT_p}{L}\right) \quad (19)$$

which leads to the absolute upper limit

$$\alpha \leq \left(\frac{L}{cT_p}\right)^{1/2} \left(\frac{\bar{v}^2}{c^2}\right)^{1/3} \left(\frac{\lambda}{L}\right)^{1/6} \quad (20)$$

Substituting the values $L/cT_p \approx 10^{-8}$ and $\bar{v}^2/c^2 \approx 10^{-5}$ we find that for the most efficient imaginable case, that of a roughly 10^6 solar mass binary source at a distance of a few tens of parsecs, we shall have

$$\alpha \leq 10^{-7} \left(\frac{\lambda}{L}\right)^{1/6} \quad (21)$$

Even on the implausible assumption that $10^6 M_\odot$ black holes constitute a substantial fraction of the mass of our Galaxy (whereas realistically one would expect at most one or two in the nucleus), it would be extremely unlikely that a pair would occur within tens of parsecs of the Solar System. Moreover, even if it did exist the *a priori* likelihood that it would be emitting its final burst of gravitational radiation at just our epoch in the history of the Galaxy would still be minute. But even if (as a result of an anthropic selection effect?!) such a system actually were present now, the maximum amplitude allowed by equation (21) for the excitation of 160-min solar oscillations (for which $\lambda/L \approx 10^{-4}$) would be at the most of the order of 10^{-8} , which is far below the observed value $\alpha \approx 10^{-5}$.

We conclude, therefore, that these particular oscillations cannot be due to gravitational radiation from any conceivable binary source, at least within the framework of standard gravitational

theory. Indeed, as the preceding arguments depend on little more than merely dimensional considerations, one can expect that the corresponding energetic restrictions will rule out even very exotic gravitational radiation emission mechanisms, including those that might be imagined in theories more general than that of Einstein.

Received 31 October; accepted 30 December 1983.

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Gravitational wave excitation of the 160-min solar oscillation?

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Recent γ -ray observations¹ of the, apparently, nearby source Geminga suggest the possibility that it is a close binary pair of compact stellar objects orbiting with a period of 160 or perhaps 320 min (Delache¹ speculates that a 160-min periodicity in the γ -ray flux might still be a signature of a 320-min orbital period). The coincidence of this period with the observed 160-min periodicity in the full disk solar velocity data has led G. Isaak (personal communication) and others to speculate that the gravitational waves emitted by the binary pair are exciting a normal mode of the Sun. Solar velocity data^{2–4} confirm the existence of an approximately 50 cm s⁻¹ oscillation in integrated Doppler shift observations that has been phase coherent for ~ 9 yr. We report here a calculation of the solar cross-section for gravitational wave excitation and discuss why such a mechanism is an extremely unlikely explanation of the observed solar oscillation.

Knowledge of the relevant solar eigenmode and corresponding damping time can be used to calculate the Sun's resonant gravitational wave cross-section. While there is some uncertainty in the solar model, the calculated structure of the eigenmodes should be sufficiently accurate. Theoretical μ -mode eigenfrequency calculations⁵ typically disagree with observations⁶ by no more than a few parts in a thousand. Furthermore, there are claims⁷ that g -modes have been observed having frequencies in agreement with model calculations to five parts per thousand.

We have used the 'Sacramento Peak Observatory Pulsation Code' to calculate eigenmodes near 160-min periods (see, for example, ref. 8). Our solar model has a central density of 147 g cm⁻³, mixing length to scale height ratio $\ell/H = 1.5$, $Z = 0.02$, and $Y = 0.24$.

Gravitational waves are of helicity two and can only couple to $\ell = 2$ solar distortions. From numerical calculations we calculate that the $\ell = 2$ mode nearest to the observed 160-min oscillation is the g_9 gravity mode.

Long damping times for these modes could make them potentially interesting as resonant gravitational wave detectors. In fact the damping time is long because the g -modes are efficiently confined to the interior where the radiative diffusion time scales are large. Our nonadiabatic calculation yields a decay time, τ , of 4×10^4 yr for the g mode at frequency $\omega = 6.65 \times 10^{-4}$ s⁻¹. These calculations are consistent with the mode lifetimes determined by Dziembowski⁹.

The solar normal mode is driven by a sinusoidally varying strain induced by the propagating gravitational wave. The effective force density induced by a wave propagating along the y

axis in spherical coordinates has the form

$$\vec{f}(r, \theta, \phi, t) = \rho \frac{\omega^2}{4} e^{i\omega t} \vec{\nabla} \left\{ \left(\sqrt{\frac{2\pi}{15}} (Y_{22} + Y_{2\bar{2}}) - \sqrt{\frac{4\pi}{5}} Y_{20} \right) h_+ + \sqrt{\frac{8\pi}{15}} (Y_{2-1} - Y_{21}) h_x \right\} r^2 \quad (1)$$

where Y_{2m} are normalized spherical harmonics, ω is the angular frequency of the wave, and h_+ and h_x are the dimensionless amplitudes of the two wave polarization components¹⁰. The g -modes have amplitude structures given by

$$\vec{\xi}_{2m}(r, \theta, \phi, t) = \{ Y_{2m} \xi_1(r) \hat{r} + \xi_2(r) \vec{\nabla} Y_{2m} \} e^{i\omega t} e^{-t/\tau} \quad (2)$$

where $\xi_1(r)$ and $\xi_2(r)$ give the radial dependence of the radial and transverse displacement amplitudes, respectively. The steady-state amplitude of the driven oscillation is readily calculated by expanding the driving force in terms of the solar normal modes. The $m=0$ mode is excited with the greatest amplitude for this geometry. As the 50 cm s^{-1} amplitude is derived from full disk velocity data, the implied $m=0$ mode amplitude is larger than this by a factor determined from the spatial velocity instrumental response function. We obtain

$$h_+^2 = \frac{5}{\pi} \langle v^2 \rangle \frac{1}{(\tau\omega)^2} S_1 S_2 R_S^{-2} \quad (3)$$

where $\langle v^2 \rangle$ is the observed full disk velocity amplitude squared; $S_1 = 1.6$ is the dimensionless instrumental response to an $\ell=2$, $m=0$ g -mode¹¹; $S_2 = 7.6 \times 10^3$ is a dimensionless 'matrix element' for the driving force-mode overlap; and R_S is the solar radius. Equation (3) yields a dimensionless wave amplitude of $h_+ = 1.6 \times 10^{-13}$, corresponding to an energy flux density of

$F = 9.6 \times 10^4 \text{ erg cm}^{-1} \text{ s}^{-1}$ (if we assume a comparable h_x polarization component). This mode has an energy $E = 6 \times 10^{28} \text{ erg}$. If we define the solar gravitational wave cross-section, σ , by $\tau\sigma F = E$, we obtain $\sigma = 5 \times 10^{11} \text{ cm}^2 = 3 \times 10^{-11} (\pi R_S^2)$.

This is an 'unreasonably' large energy flux. Two equal masses in circular orbits (of mass M solar masses and distance d light years from the Sun) must satisfy $M^{5/3}/d > 2 \times 10^5$ to produce this energy flux at the Sun¹⁰. It is extremely unlikely that Geminga satisfies this criterion. Given that it does, it is even more unlikely that the resonant condition will be satisfied long enough for the mode to build up to this amplitude. As the g -mode Q value is so high, frequencies must coincide to within a few parts in 10^9 for at least one damping time of $\sim 4 \times 10^4 \text{ yr}$. Unless the radiating system is in the immediate solar neighbourhood, the total energy radiated during this time must be a significant fraction of the total kinetic energy of the system. It is difficult to see how the orbital frequency could remain tuned to the solar resonance for long in these circumstances.

We thank P. Goode and W. Dziembowski for interesting discussions. The research was supported by NSF grant PHY 8118157.

Received 5 December; accepted 30 December 1983.

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The Sun as a detector of gravitational waves

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One possible explanation of the solar 160-min oscillations discovered by Severny *et al.*¹ and Brookes *et al.*² is that they may be due to excitation by gravitational waves emitted from a nearby binary system. Here we demonstrate that even in the most favourable conditions, the absorption rate of gravitational radiation by the Sun is many orders of magnitude less than is required to overcome the effect of damping in the core.

The observed 160-min oscillations have been interpreted as surface manifestations of low- ℓ g -modes (ℓ is the degree of the spherical harmonic $Y_{\ell m}$) which are trapped below the convection zone in the solar core (for a review of the properties of these modes, see refs 3, 4). The driving mechanism responsible for the excitation of these g -modes has not yet been identified. Radiative damping in the solar core is the primary cause of the decay of the pulsation. Keeley⁵ has found that a quadrupole ($\ell=2$) g_{10} mode with a period near 160 min will be damped at a rate $\kappa = 4.3 \times 10^{-13} \text{ s}^{-1}$. The observed radial velocity amplitude of $\sim 1 \text{ m s}^{-1}$ (refs 1, 2) implies a pulsation energy $E_p = 5.8 \times 10^{36} \text{ erg}$ for Keeley's quadrupole g_{10} mode.

We now ask whether gravitational waves emitted from a nearby binary system can supply this mode with sufficient energy to excite or maintain the oscillations. As a generator of gravitational radiation, we consider a binary system consisting of two objects, of mass M_1 and M_2 , in relative circular orbit with an

orbital period $P_b = 320 \text{ min}$. (Note that the frequency of the gravitational waves is twice the orbital frequency⁶.) The assumption of a circular orbit allows the gravitational wave energy to be concentrated at a single frequency; a non-circular orbit would distribute the energy throughout the higher harmonics⁶. In the weak-field, slow-motion limit, the quadrupole gravitational wave luminosity is⁷

$$L_b = \frac{32}{5} \frac{G}{c^5} \frac{M_1^2 M_2^2}{M_1 + M_2} r^4 \omega_b^6 \quad (1)$$

where r is the separation of the two objects and $\omega_b = 2\pi/P_b$. For a keplerian orbit, this becomes

$$L_b = \frac{32}{5} \frac{G^{7/3}}{c^5} M_1^2 M_2^2 (M_1 + M_2)^{-2/3} \omega_b^{10/3} \\ = 1.1 \times 10^{31} \text{ erg s}^{-1} \left(\frac{M_1}{M_\odot} \frac{M_2}{M_\odot} \right)^2 \left(\frac{M_1 + M_2}{M_\odot} \right)^{-2/3} \quad (2)$$

The radiation is not emitted isotropically; the time-averaged distribution, summed over polarization states, is proportional to $(1 + 6 \cos^2 \theta + \cos^4 \theta)$, where θ is the angle between the observer and the angular momentum vector of the binary system⁶. However, the angular variation is less than an order of magnitude, and we ignore it in what follows. If the binary system is $D \text{ pc}$ from the Sun, the flux of gravitational radiation arriving at the Sun is

$$F = 9.5 \times 10^{-8} \text{ erg cm}^{-2} \text{ s}^{-1} \left(\frac{M_1}{M_\odot} \frac{M_2}{M_\odot} \right)^2 \\ \times \left(\frac{M_1 + M_2}{M_\odot} \right)^{-2/3} \left(\frac{1 \text{ pc}}{D} \right)^2 \quad (3)$$

We next consider the absorption of this radiation by the Sun. The quadrupole oscillation of a non-rotating, spherically symmetric star consists of $2\ell + 1 = 5$ degenerate modes. However, in the presence of rotation or magnetic fields, this m degeneracy

is broken⁸. We shall examine the extreme (and undoubtedly unrealistic) situation where the gravitational waves couple with only one of these modes, in particular a single φ_{10} mode. (The choice of spherical harmonic index m is unimportant; for $\ell = 2$, our results are the same for all values of m .) We further assume that the solar core (where the trapped φ -modes reside) is 'tuned' to resonate at $\omega_g = 2\omega_b$, the frequency of the gravitational waves. This is a generous assumption because the Sun is a "high- Q " oscillator with a narrow bandwidth ($\Delta\omega/\omega_g \approx 6.6 \times 10^{-10}$). The frequency of the gravitational waves will quickly pass through this bandwidth, with a timescale of years for $M_1, M_2 \approx 1 M_\odot$, as the period of the binary system decreases with the emission of the gravitational radiation. The Doppler shift caused by the Sun's motion about the barycentre of the Solar System will further serve to shift the frequency of the gravitational radiation outside the solar bandwidth. Nevertheless, we ignore these effects in the following analysis.

A calculation of the absorption cross-section for gravitational waves by the φ_{10} mode requires an estimate of the damping rate due to re-emission (scattering) of the absorbed radiation. The quadrupole formula for the gravitational wave luminosity gives¹⁰

$$L_g = \frac{GM_\odot^2 R_\odot^4}{12\pi c^5} \omega_g^6 \left(\frac{4\pi}{5} \frac{1}{M_\odot R_\odot^2} \int_0^{R_\odot} r^4 \rho'(r) dr \right)^2 \quad (4)$$

where ρ' is the (eulerian) variation in the density produced by the oscillation. The quantity in square brackets has been calculated by Christensen-Dalsgaard and Gough¹¹. Using their results for an $\ell = 2$ mode with a period of 157 min yields $L_g = 1.7 \times 10^{15} \text{ erg s}^{-1}$ for a mode with the observed surface velocity amplitude of $\sim 1 \text{ m s}^{-1}$. The damping rate due to re-radiation is then $\kappa_g = L_g/E_p = 3.0 \times 10^{-22} \text{ s}^{-1}$. The mechanical damping rate κ dominates this value by nine orders of magnitude. Thus, any scattering of the incoming gravitational waves may be ignored. The absorption cross-section, σ , for our 'tuned' solar detector is then approximately¹²

$$\sigma = \frac{\kappa_g}{\kappa} \left(\frac{2\pi c}{\omega_g} \right)^2 = 5.8 \times 10^{19} \text{ cm}^2 \quad (5)$$

This is less than 0.4% of the Sun's geometrical cross-section.

We can now estimate the ratio of the gravitational wave energy absorbed by the Sun over one damping time to the pulsation energy E_p :

$$\frac{1}{\kappa} F\sigma/E_p = 2.2 \times 10^{-12} \left(\frac{M_1}{M_\odot} \frac{M_2}{M_\odot} \right)^2 \left(\frac{M_1}{M_\odot} + \frac{M_2}{M_\odot} \right)^{-2/3} \times \left(\frac{1 \text{ pc}}{D} \right)^2 \quad (6)$$

The inescapable conclusion is that even in the most favourable conditions, the 160-min oscillations cannot possibly be driven by gravitational waves emitted by a nearby binary system.

We thank H. M. Van Horn and M. P. Savedoff for helpful suggestions. This research was partially supported by NSF grant AST 83-07214.

Received 7 November; accepted 30 December 1983.

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Beam structure of Jupiter's decametric radiation

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The well-defined zones of central meridian longitude within which the probability of jovian radio emission at frequencies near 22 MHz is relatively high are known as sources A, B and C. Each consists of a component for which the emission probability is strongly correlated with Io's orbital position, and another component that is Io-unrelated¹. This emission anisotropy and other observed effects have long been interpreted in terms of quasi-continuous beams that co-rotate with the inner jovian magnetosphere. Observations made from the Soviet spacecraft Mars 7 and the Nancay Observatory² provided more direct evidence for beaming; activity observed from one of the stations was not detectable from the other. We now present convincing evidence based on concurrent observations from the two Voyager spacecraft and a terrestrial observatory that the component of source A radiation that is not correlated with Io's position is generally emitted in co-rotating 'searchlight beams' of distinctive cross-sectional shape.

Simultaneous observations of Jupiter were made in early 1979 at 21.811 MHz (200 kHz bandwidth) from Voyagers 1 and 2, and at 21.860 MHz (13 kHz bandwidth) from the Mizuho-cho Radio Observatory in Japan. The Voyager 1 and Mizuho-cho records were compared, and 13 noise storms were found which exhibited a high degree of correlation at the two stations. Essentially all of these correlated storms were of type non-Io-A (independent of Io's orbital position), following the classification presented in Table 7.4 of Carr, Desch and Alexander¹. As the distance to Jupiter decreased during the observation period, the relative detection sensitivity of the spacecraft receiver increased rapidly. To minimize the effect of this sensitivity change, five correlated storms occurring over the relatively short interval from 31 January to 10 February were selected from the total of 13 for the present analysis. As we shall show, the ratios of peak Jupiter intensity to minimum detectable signal level for the Voyager 1 and Mizuho-cho radiometers were approximately the same for the five selected storms. The time spans during which the strongest storms were observed by Voyager 2 and by the ground station did not overlap, but we were able to identify two of the five selected storms on the recordings from Voyager 2 as well as on those from Voyager 1 and Mizuho-cho. Although several Io-B storms (that is, the particularly powerful storms representing the source B component that is strongly correlated with Io's orbital position) were also observed, they were much more poorly correlated on the Voyager and ground station records than were the non-Io-A storms, indicating pronounced differences between non-Io-A and Io-B beam characteristics. Some of the intense Io-B storms observed at Mizuho-cho were not detected at all by the Voyagers. This result, which suggests that the Io-B beam is particularly narrow and may change its shape within times of the order of half an hour, agrees with the earlier spacecraft-ground station results of Poquerusse and

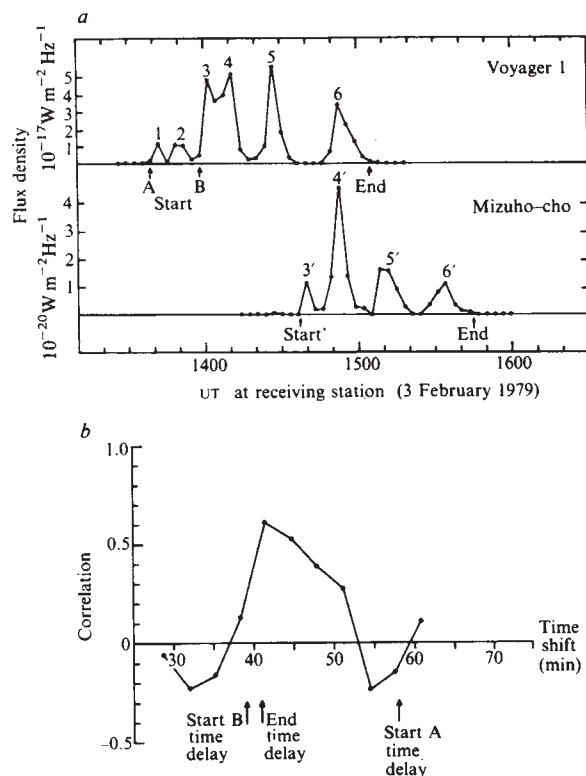


Fig. 1 Comparison of the same jovian storm (at ~ 22 MHz) as observed from the Voyager 1 spacecraft and the Mizuho-cho Observatory in Japan. *a*, Flux density against time at the two stations (3.2 min averaging interval). Peaks 1–6 (upper curve) were due to separate spectral arcs that were clearly discernible in the corresponding Voyager 1 dynamic spectral plot. Peaks 3'–6' (lower curve) are believed to be later manifestations at the terrestrial station of the same arcs responsible for peaks 3–6 in the spacecraft data. *b*, Cross-correlation of the two curves, the time shift being that of the upper curve with respect to the lower.

Lecacheux². The storms they observed, which were of type Io-B only, could be detected at one or the other of the two stations but not at both.

Figure 1a shows plots of flux density against time for one of the selected storms as received at Voyager 1 and at the Mizuho-cho Observatory. The cross-correlation of the two curves as a function of the time shift of the upper one relative to the lower is plotted in Fig. 1b. The peak correlation, 0.62, occurs at a shift of about 42 min. We believe that this relatively high correlation strongly supports the assumption that each curve in Fig. 1a resulted from the rotation of the same more or less stable complex emission beam pattern past the direction of the observing station, the beam rotating with the inner jovian magnetosphere at the System III (1965) angular velocity. The 42-min time difference (occurrence time at Earth minus that at Voyager 1) is the algebraic sum of three components. These are the difference in propagation times from Jupiter to the receiving stations (+34 min), the difference in right ascensions of the stations divided by the System III angular velocity of rotation (+38 min), and a third component (–30 min) due to the combined effect of the shape of the beam cross-section and the difference in station declinations. It is the last component that provided the information used in our analysis of the beam structure.

The more usual way of displaying Voyager Jupiter data has been by means of the dynamic spectrum, in which signal intensity is indicated by shades of grey on the frequency–time plane. A decametric storm on such displays is generally made up of a family of nested arcs, like a series of opening or closing parentheses^{1,3,4}. The Voyager 1 dynamic spectral plot of the storm of which the upper part of Fig. 1a is a fixed frequency profile

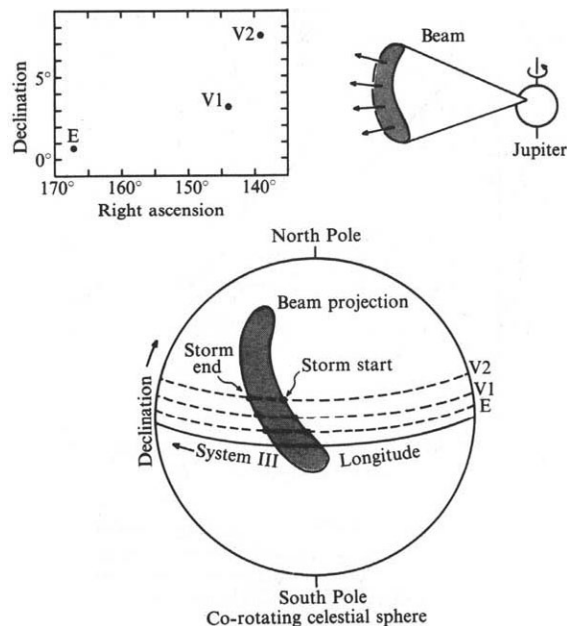


Fig. 2 Beaming geometry. At upper left are shown the relative positions, in terms of jovicentric sidereal coordinates, of Earth, Voyager 1 and Voyager 2 in early February 1979. The sketch at the upper right suggests the shape of the assumed emission beam envelope that rotates with Jupiter. The co-rotating celestial sphere, at the bottom, indicates qualitatively the System III longitudes and declinations of the three stations at the start and end of the storm produced by the passage of the beam envelope.

shows well-defined spectral arcs corresponding to peaks 1–6. These arcs were of the closing-parenthesis, vertex late shape characteristic of non-Io-A storms¹. Their vertex frequency (that is, the frequency at which a constant-time line is tangent to each arc) was about 12 MHz. It seems likely that peaks 3'–6' in the lower plot are due to the same arcs as are peaks 3–6. The difference in shapes and relative heights of the corresponding peaks, and the absence of peaks 1' and 2', could be due entirely to the effects of scintillation and variable Faraday rotation in the terrestrial ionosphere above the Mizuho-cho Observatory, or alternatively they could be due to a combination of terrestrial ionospheric effects and actual differences in the emitted beam intensity at the different declinations and times. The start and end times of the correlated parts of the storm at the two stations were estimated (using 'start B' for the Voyager 1 curve), and the delays of one start time relative to the other and of one end time relative to the other are indicated by arrows in Fig. 1b. It is apparent that both delays are close to that at which the peak correlation for the entire curves occurs.

It was not feasible to obtain cross-correlation plots for determining relative station delays for the other members of the group of five selected storms. We have instead used storm start and stop times in the analysis to be described below. The 18-min difference between 'start A delay' and 'start B delay' in Fig. 1b is indicative of the error that can be made in using this method when a storm builds up or decays in intensity gradually rather than abruptly. To minimize such errors, it is important that the relative sensitivities of the receiving equipment at the spacecraft and ground stations be approximately the same. Fortunately, this was true in the case of our group of five selected storms. We have estimated peak Jupiter flux density, background noise level and signal-to-noise ratio for the two recordings in Fig. 1a. The peak flux densities received at Voyager 1 and at the Mizuho-cho Observatory were 5×10^{-17} and $5 \times 10^{-20} \text{ W m}^{-2} \text{ Hz}^{-1}$, respectively. The Voyager 1 value adjusted to Earth distance is $11 \times 10^{-20} \text{ W m}^{-2} \text{ Hz}^{-1}$, which is about 3 Db higher than the terrestrially measured value. The mean of the flux densities of peaks 3, 4, 5 and 6 in the Voyager 1 curve, adjusted to Earth

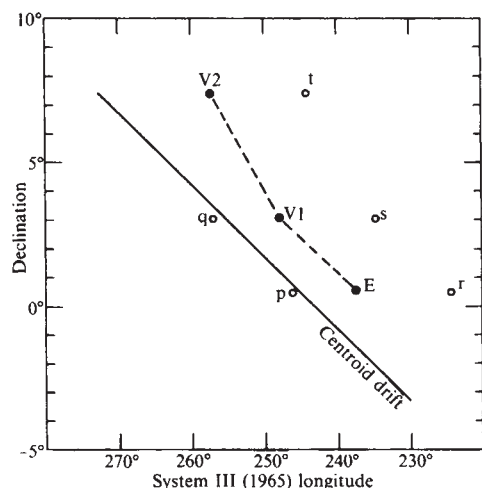


Fig. 3 Station positions at starting and ending times of correlated storms, plotted on the celestial sphere that co-rotates with Jupiter. E, V1 and V2 indicate the mean declinations of Earth, Voyager 1 and Voyager 2 (0.55° , 3.15° and 7.41°), respectively. Each solid line connects points representing the station positions at the start of the storm designated by the letter at the top of the line, and the dashed line labelled with the same letter connects the station points at the end of that storm.

distance, is about 6 Db higher than the mean of peaks 3', 4', 5' and 6' of the Mizuho-cho curve. Although these differences are no greater than would be expected from the Voyager radiometer calibration uncertainties, they could also be attributed to terrestrial ionospheric effects in the Mizuho-cho recording. The background noise level of the Voyager receivers was mainly due to interference from within the spacecraft, while that for the Mizuho-cho receiver was mainly galactic noise. The minimum detectable flux densities for the Voyager 1 and Mizuho-cho radiometers as calculated from background levels, bandwidths and integration times were estimated to be about 10^{-18} and $3 \times 10^{-22} \text{ W m}^{-2} \text{ Hz}^{-1}$, respectively. The corresponding ratios of peak Jupiter flux density to minimum detectable flux density were of the order of 10^2 for both radiometers.

In the analysis of the group of five selected non-Io-A storms, the start and end of each storm were treated as separate events. It is assumed that each such event at each station resulted from the passage of the leading or trailing edge of an emission beam that co-rotates with the inner magnetosphere at the System III angular velocity. The propagation delays to the observing stations were unequal, and were continually changing. In this analysis the propagation delay was subtracted from the time of observation at each station, so that the corrected time referred to the instant at which the observed radiation left the planet. Differences in the jovicentric right ascensions of the stations were taken into account by converting each corrected event time into the System III central meridian longitude at that instant, as viewed from the jovicentric right ascension of the corresponding station. Any significant differences in the central meridian longitudes of a given event at the two or three stations could thus be attributed to the combined effect of the shape of the beam cross-section and the differences in station declination. The jovicentric right ascension and declination of each station in early February 1979 are indicated in Fig. 2. The declinations were essentially constant throughout the observation period, the mean values being $+0.6^\circ$ for Earth (E), $+3.1^\circ$ for Voyager 1 (V1) and $+7.4^\circ$ for Voyager 2 (V2).

The situation is simplified conceptually by considering projections onto a co-rotating celestial sphere on which the coordinates are jovicentric declination and System III longitude, as shown in Fig. 2. A given observed event is represented by a point on this sphere at the declination of the observing station and at a longitude equal to the central meridian longitude from the

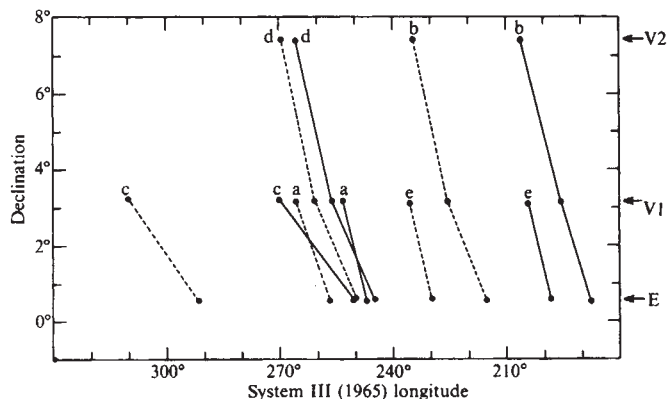


Fig. 4 Mean positions of the three stations at the midpoints of the correlated storms (filled circles) compared with published data on the long-term variation of the non-Io-A longitude centroid with the jovicentric declination of the observer (straight line). Points p and q are the mean midpoints for the two-station correlated storms; r, s and t are those for three stations. Points E and V1 are the means for all the storms. Point V2 was determined by moving segment st parallel to itself until s coincided with V1.

station at the propagation-corrected event time. Figure 3 shows the points corresponding to the two events (start and end) defined by each storm that was observed at each station. Although the peaks corresponding to individual spectral arcs within storms were often not clearly distinguishable in our single-frequency plots, the measured start and end times for each station were presumably the beginning of the first detectable arc and the ending of the last one, respectively. In Fig. 3 the solid lines connect corresponding points representing the start of a storm, and the dashed lines connect the ending points. The region between corresponding solid and dashed lines indicates the directions in which radiation was being emitted; it therefore represents a portion of the cross-section of the envelope of the storm beam structure (that is, the envelope of the narrow beams associated with the individual spectral arcs comprising the storm). Several features of Fig. 3 should be noted. The leading and trailing boundaries of the beam envelope for each storm were inclined in such a way that they encountered lower-declination stations before higher-declination ones. The two boundaries of every beam envelope appear to have been roughly parallel to each other and to those of the others. The slopes ($\Delta \text{dec.}/\Delta \text{long.}$) of the segments E to V1 in Fig. 3 average 0.31 for storm beginnings and 0.27 for storm endings, while for the V1 to V2 segments the averages are 0.43 and 0.49, respectively.

These results agree qualitatively with a model in which the storm emission beam envelope is in the form of a curved sheet that co-rotates with the planet, as suggested by Fig. 2. If it is further assumed that the leading and trailing boundaries of the curved emission beam are cones, that the axes of the cones are aligned with the directions of the magnetic field in the source region, and that the emission frequency is close to the electron cyclotron frequency, it is possible to infer the jovigraphic latitude and longitude of the non-Io-A source. Our preliminary calculations based on the O_4 magnetic field model⁵ suggest that this source lies in or near the northern jovian auroral zone. If the boundaries of the emission beams were full cones, there would be another non-Io emission region at central meridian longitudes on the opposite side of the longitude towards which the jovian magnetic dipole vector is tipped ($\sim 200^\circ$), with storm beginning and ending slopes opposite to those of the lines in Fig. 3. A search revealed no such image source. It was therefore necessary to assume that the emission beam must be approximated by a sector of a cone, as in Fig. 2, rather than by a full cone.

Finally, we examine these results in the light of long-term statistical studies of source non-Io-A based on two decades of terrestrial observations⁶⁻⁹ and the more recent general

catalogues of Voyager Jupiter activity (that is, events listed without regard to possible ground station correlations)¹⁰. In Fig. 4 the points E, V1 and V2 represent the non-Io-A storm longitude centroid for each declination, based on the data from our five selected correlated storms. The long straight line is a fit to all the available published data, including the long-term terrestrial observations and the catalogues of Voyager Jupiter activity⁷⁻¹⁰. The agreement of our three points with this line is relatively good, especially with regard to slope. We conclude that the well-known quasi-linear relationship between the centroid of the long-term longitude distribution of non-Io-A occurrence probability and the joventric declination of the observer is apparently a result of the superposition of the effects of a great many co-rotating but relatively short-lived emission beams occurring throughout the source A region of central meridian longitude, most of which have the characteristic shape suggested by our model. This shape, a curved sheet that is concave on its higher longitude side, is in qualitative agreement with the conical sheet beams that were assumed in some models^{3,11-13} accounting for the decametric spectral arcs when such models are applied to source non-Io-A.

We thank N. Oda for assistance in making observations, and J. J. Schauble for providing Voyager antenna calibration data. T.D.C. was supported by NASA through grant NAGW-320 and by NSF through grant AST 8000246.

Received 17 June; accepted 1 December 1983.

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Ion microprobe magnesium isotope analysis of plagioclase and hibonite from ordinary chondrites

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The finding of abundant aluminium-rich objects within ordinary chondrites¹⁻³ supports the suggestion⁴ that carbonaceous and type 3 ordinary chondrites, and their components⁴⁻⁷, share common origins. However, oxygen isotope systematics⁸ demonstrate that the chondrules present within the two meteorite types have isotopically distinct solid precursors, so while they may have a similar mode of formation, the chondrules come from isotopically separate reservoirs (a later reaction with a similar ambient gas is a possibility⁸). ²⁶Mg excesses generated by the *in situ* decay of ²⁶Al have previously only been found within Ca-Al-rich inclusions (CAIs) from carbonaceous chondrites⁹⁻¹⁴. In a search for ²⁶Mg excesses generated by ²⁶Al decay we analysed four Al-rich objects from the type 3 ordinary chondrites using an ion microprobe. We report here the presence of ²⁶Mg excesses of up to 100% in an unusually pure

hibonite clast from the Dhajala chondrite; this ²⁶Mg excess is the first to be found in an ordinary chondrite. Despite the large ²⁶Mg excesses, the initial ²⁶Al/²⁷Al ratio defined (8.4×10^{-6}) is some five times lower than that commonly observed in calcium-aluminium-rich inclusions from the carbonaceous chondrites. No ²⁶Mg excesses were observed in the three plagioclase-bearing chondrules analysed. The presence of ²⁶Mg excesses within both unequilibrated ordinary and carbonaceous chondrites supports a common origin for components of these two meteorite types; however, ²⁶Al may not have been sufficiently plentiful to act as a major heat source in condensed Solar System bodies¹⁵⁻¹⁷.

No ²⁶Mg excesses were observed in an earlier study of plagioclase from more evolved meteorites including aubrites, eucrites, type 6 ordinary chondrites and irons¹⁵. ²⁶Mg excesses (if originally present) are, however, more likely to be preserved in type 3 ordinary chondrites which have undergone much less reprocessing after accretion. In this study mineral compositions (Table 1) were determined using an ARL EMX-SM electron microprobe operated at 15 kV accelerating potential and a 20 nA sample current; analyses were corrected by standard procedures¹⁸. The Ca-Al-rich objects were also studied with Hitachi 450 and JEOL JSM-35 scanning electron microprobes (SEMs) operated at 15-25 kV and equipped with energy dispersive analysers. The magnesium isotopes were measured, at low resolution, on the Chicago AEI IM 20 ion microprobe using the techniques and standards of Hutcheon¹⁴ and Bar-Matthews *et al.*¹⁹. Corrections were made for the unresolved overlap of ⁴⁸Ca²⁺ at mass 24 using the measured ⁴⁰Ca²⁺ intensity at mass 20. ²⁷Al⁺ intensities could not be recorded during magnesium isotope analysis because of its high intensity under normal recording conditions. Within-run ²⁷Al/²⁴Mg⁺ ratios were therefore calculated using well-defined working curves of ²⁷Al/²⁴Mg⁺ against ⁴⁰Ca²⁺/²⁴Mg⁺ (separate curve for each mineral) given by ⁴⁰Ca²⁺, ²⁴Mg⁺ and ²⁷Al⁺ intensities recorded at the beginning and end of each run. Absolute ²⁷Al/²⁴Mg ratios are estimated to be $\pm 8\%$ (2σ) for individual analyses and $\pm 10\%$ for within-run measurements (see below). The ²⁵Mg/²⁴Mg ratios recorded for the hibonite standard were fractionated relative to true values but were reproducible to $\pm 5\%$ (2σ) over the 2-month period that the data were recorded. Absolute ²⁵Mg/²⁴Mg ratios given in Table 2 assume that the discrimination for unknowns is the same as that of the standard.

The plagioclase analysed within the ALHA 77299 (H3) and Sharps (H3) chondrites occurs as constituent grains in Ca-Al-rich chondrules. Chemical analyses are given in Table 1. Plagioclase (anorthite An ~90) from ALHA 77299 occurs as

Table 1 Electron microprobe analyses (in wt%) of plagioclase and hibonite

	1	2	3†	4	5
SiO ₂	45.2	54.5	44.0	45.6	0.26
TiO ₂	0.11	<0.04	—	—	<0.05
Al ₂ O ₃	35.7	28.9	34.5	35.4	90.8
Cr ₂ O ₃	<0.04	—	—	<0.05	<0.08
FeO	0.21	1.6	1.07	0.38	0.44
MgO	<0.01	<0.02	—	<0.02	<0.02
CaO	17.7	10.9	17.75	18.1	8.9
Na ₂ O	1.14	4.8	1.47	1.15	<0.01
K ₂ O	<0.02	0.03	—	<0.01	—
Total	100.13	100.79	—	100.71	100.56
SF*	20.02	19.99	—	20.04	—
An	89.5	55.5	87.0	89.9	—
Ab	10.4	44.3	13.0	10.0	—
Or	0.1	0.2	—	0.1	—

1-4, Plagioclase; 5, hibonite; 1, ALHA 77299, 40; 2, Sharps (National Museum of Natural History, Washington DC, G40-1); 3, Krymka (Field Museum of Natural History, Chicago, ME 2753); 4, Hobbs (University of New Mexico, 45); 5, Dhajala (University of New Mexico, 127).

* Structural formula cation for feldspar analysis based on 32 oxygen atoms.

† Analysed by energy dispersive analysis on a JEOL JSM-35 SEM.

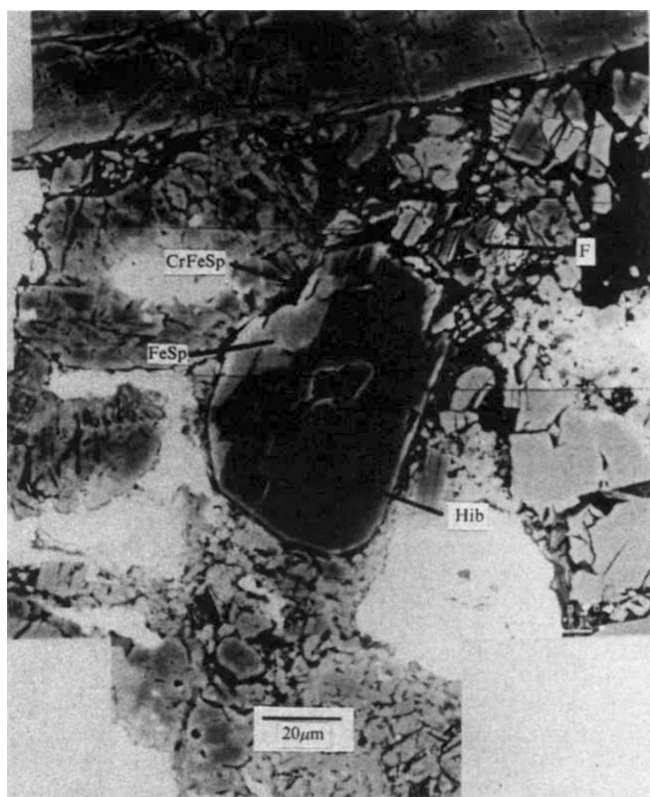


Fig. 1 Backscattered electron image of the hibonite fragment ($\sim 70 \mu\text{m}$ long) from the Dhajala (H3) ordinary chondrite using a JEOL JSM-35 SEM. The hibonite grain has been repolished after ion microprobe analysis to a depth of $\sim 5 \mu\text{m}$. The holes caused by ion microprobe analysis can be seen to be restricted to the centre of the hibonite grain. Hib, hibonite; FeSp, Fe-rich spinel; CrFeSp, Fe-Cr-rich spinel; F, fragmented hibonite and spinel. Scale bar, $20 \mu\text{m}$.

elongated laths up to $400 \mu\text{m}$ long and $60 \mu\text{m}$ wide. They are embedded in a fine-grained intergrowth of more sodic plagioclase and a Ti-bearing phase (mineral identification uncertain due to its small size). Other constituents of this chondrule include zoned spinel (FeO: 12.4–14.4 wt%; Cr_2O_3 : 1.5–11.4 wt%) and fassaite (Al_2O_3 : 8.4 wt%; TiO_2 : 4.1 wt%). The plagioclase within Sharps occurs as anhedral to subhedral crystals which are mainly restricted to the chondrule rim. The chondrule ($200 \mu\text{m}$ in apparent diameter) consists of approximately 70 vol% plagioclase; minor phases present include chromite, low-Ca pyroxene and an Fe-rich mineral, probably olivine. In the Krymka (LL3) ordinary chondrite, plagioclase (An ~ 90) is present as an interstitial phase between olivine and low-Ca pyroxene within a porphyritic chondrule. The total chondrule is not Ca-Al-rich, as it has a bulk Al_2O_3 of less than 5%.

A single hibonite clast ($\sim 70 \mu\text{m}$ long, Fig. 1) was found within the Dhajala (H3) ordinary chondrite. Electron microprobe analyses revealed that this hibonite grain is extremely pure, with contents of MgO, TiO_2 and Na_2O below the detection limits (Table 1); ion microprobe measurements gave magnesium contents of 17 to 200 p.p.m. The only previously reported hibonites of comparable purity are those found within the HAL inclusion from the Allende meteorite^{20,21}. Both hibonites contain small amounts of Fe (FeO: 0.3–0.5 wt%) and HAL hibonite also contains moderate amounts of Ti (TiO_2 : ~ 0.7 wt%). The hibonite within the centre of the HAL inclusion contains fine Ti-rich needles, inferred to be rutile; in contrast, Dhajala hibonite has a discontinuous rim of Fe-rich and Fe-Cr-rich spinel (Fig. 1). The Dhajala hibonite grain has a few well-defined crystal faces and a moderately well-developed cleavage. Repolishing after analysis revealed that the original crystal has

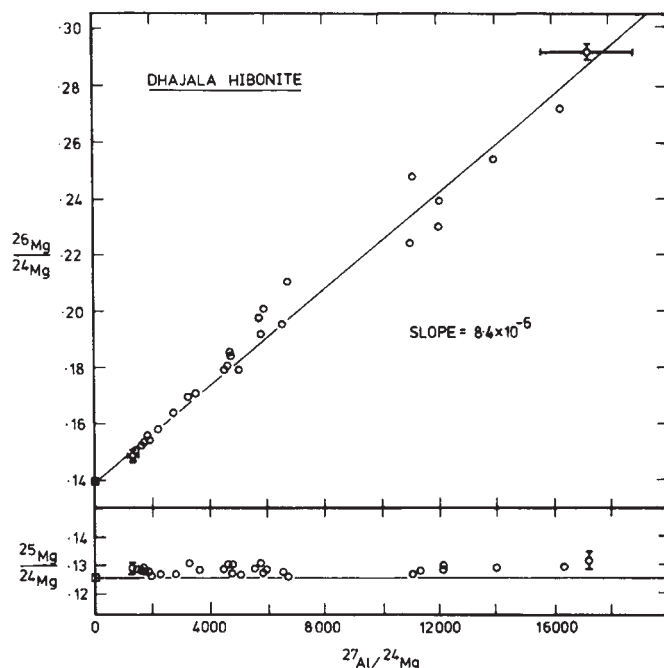


Fig. 2 $^{26}\text{Mg}/^{24}\text{Mg}$ (corrected for mass fractionation; see refs 14, 19) and $^{25}\text{Mg}/^{24}\text{Mg}$ (uncorrected) against $^{27}\text{Al}/^{24}\text{Mg}$ ratios for Dhajala hibonite (○) and the Madagascar standard (□). A slope of $8.4 \pm 0.5 \times 10^{-6}$, intercept 0.1410 ± 0.0041 (2σ) and correlation coefficient 0.9754 is given for $^{26}\text{Mg}/^{24}\text{Mg}$ against $^{27}\text{Al}/^{24}\text{Mg}$. A slope of $1.38 \pm 1.2 \times 10^{-7}$, intercept 0.12741 ± 0.00093 and correlation coefficient 0.1775 is given for $^{25}\text{Mg}/^{24}\text{Mg}$ against $^{27}\text{Al}/^{24}\text{Mg}$. The error bars are 2σ .

been fragmented at one end (Fig. 1). The HAL hibonite grain analysed in this work does not contain needles and comes from the outer clear hibonite rim.

The magnesium contents of the plagioclases analysed by ion microprobe (Table 2) varied by more than a factor of 60; however, magnesium contents of plagioclases from each chondrule only varied by a factor of 2. The average isotope ratios for the plagioclase grains within each chondrule are given in Table 2. Long runs were not made on single grains because of their small size. All plagioclase analyses were within error of the terrestrial standard and no ^{26}Mg excesses were observed outside the error limits.

As noted above, the Mg content of the Dhajala hibonite is extremely low; great care was therefore taken to exclude the possibility of overlapping the peripheral areas of the primary beam onto the included spinel or the (high Mg) olivines within the surrounding matrix. Despite the fact that only the central area of the hibonite was analysed (Fig. 1), large variations in the $^{27}\text{Al}/^{24}\text{Mg}$ ratio were recorded. The measured $^{25}\text{Mg}/^{24}\text{Mg}$ ratios were consistently greater than that recorded for the hibonite standard (averaging +19%), and even after correction for mass fractionation (see ref. 14) very high ^{26}Mg excesses were recorded. Although the isotope measurements were made at low resolution, examination of the mass spectrum at a resolution of $>1,500$ confirmed that the excess at mass 26 is not caused by the possible, if unlikely, interferences C_2H_2^+ , BO^+ , CaC^{2+} , or $^{52}\text{Cr}^{2+}$, nor to $^{27}\text{Al}^+$ scattering. The validity of the low resolution recording technique at low Mg concentrations for both ^{25}Mg and ^{26}Mg is also demonstrated by the normal values obtained on the HAL hibonite (see below and Table 2). The +19% per mass unit fractionation and ^{26}Mg excesses would therefore appear to be real.

To establish the overall range in $\delta^{26}\text{Mg}$, all Dhajala hibonite runs were divided into sections of 10 individual recordings of the Mg isotopes (runs normally comprise between 30 and 100 individual recordings). A plot of $^{26}\text{Mg}/^{24}\text{Mg}$ and $^{25}\text{Mg}/^{24}\text{Mg}$ against $^{27}\text{Al}/^{24}\text{Mg}$ for these 10 pass sections is given in Fig. 2.

Table 2 Ion microprobe magnesium isotope analysis of plagioclase and hibonite from chondrites and terrestrial standards

Meteorite	Run	$^{25}\text{Mg}/^{24}\text{Mg}^*$	$^{26}\text{Mg}/^{24}\text{Mg}$	$\delta^{26}\text{Mg}$	$^{27}\text{Al}/^{24}\text{Mg}$	$\delta^{25}\text{Mg}^\dagger$	$\delta^{26}\text{Mg}$	Phase
77299	1	0.12704 $\pm$ 172	0.14005 $\pm$ 300	-1.2 $\pm$ 25	1,860	+1	-3.0 $\pm$ 11	Plag.
	2	0.12680 $\pm$ 242	0.13979 $\pm$ 170	+0.4 $\pm$ 27	2,895			
	3	0.12706 $\pm$ 70	0.13932 $\pm$ 130	-7.4 $\pm$ 11	1,211			
Sharps	1	0.12666 $\pm$ 96	0.13869 $\pm$ 142	-5.4 $\pm$ 13	234	-4	+3.6 $\pm$ 8.1	Plag.
	2	0.12629 $\pm$ 90	0.13871 $\pm$ 140	+0.2 $\pm$ 12	136			
	3	0.12618 $\pm$ 128	0.14121 $\pm$ 181	+14.7 $\pm$ 16	119			
Krymka	1	0.12712 $\pm$ 62	0.13963 $\pm$ 90	-4.1 $\pm$ 8	44	-3	-1.3 $\pm$ 4.1	Plag.
	2	0.12582 $\pm$ 50	0.13795 $\pm$ 82	1.7 $\pm$ 7	61			
	3	0.12610 $\pm$ 56	0.13825 $\pm$ 74	-0.3 $\pm$ 7	56			
	4	0.12684 $\pm$ 44	0.14002 $\pm$ 76	1.5 $\pm$ 6	69			
Dhajala	1	0.12840 $\pm$ 174	0.17149 $\pm$ 202	204 $\pm$ 19	2,176	+19	+380	Hib.
	2	0.12709 $\pm$ 132	0.15418 $\pm$ 132	99 $\pm$ 14	1,805			
	3	0.12775 $\pm$ 198	0.18529 $\pm$ 208	313 $\pm$ 22	4,829			
	4	0.12778 $\pm$ 78	0.15089 $\pm$ 126	66 $\pm$ 9	1,742			
	5	0.12866 $\pm$ 156	0.19484 $\pm$ 404	368 $\pm$ 17	5,599			
	6	0.13021 $\pm$ 240	0.27493 $\pm$ 854	920 $\pm$ 26	15,655			
	7	0.12709 $\pm$ 294	0.20174 $\pm$ 480	441 $\pm$ 33	6,300			
	8‡	0.12871 —	0.23147 —	630 ($\pm$ 28)	10,639			
Hal	1	0.12756 $\pm$ 10	0.13748 $\pm$ 17	-0.7 $\pm$ 1.5	—	-2	+1.2 $\pm$ 3.8	Hib.
	2	0.12650 $\pm$ 10	0.13939 $\pm$ 20	+2.0 $\pm$ 1.6	—			
	1	0.12553 $\pm$ 34	0.13743 $\pm$ 36	+2.3 $\pm$ 3.8	3,176			
	2	0.12592 $\pm$ 72	0.13715 $\pm$ 80	-5.4 $\pm$ 8	9,227			
	3	0.12546 $\pm$ 78	0.13752 $\pm$ 82	+4.0 $\pm$ 9	10,704			
Standard	Analyses	$^{25}\text{Mg}/^{24}\text{Mg}^\S$	$^{26}\text{Mg}/^{24}\text{Mg}$	$\delta^{26}\text{Mg}$	$^{27}\text{Al}/^{24}\text{Mg}$			Phase
Madagascar hibonite	10	0.12583 $\pm$ 70	0.13778 $\pm$ 151	0.3 $\pm$ 3.2	352			Hib.
Lake County	9	0.12685 $\pm$ 110	0.13979 $\pm$ 191	-0.4 $\pm$ 5.5	221			Plag.
Loire Valley	3	0.12607 $\pm$ (72)	0.13837 $\pm$ (157)	+1.0 $\pm$ (1.2)	—			Spinel

* All runs are at least 30 individual recordings of the magnesium isotopes. All errors are 2σ .

† ^{26}Mg errors relative to standard $\pm 5\%$.

‡ Average of four short runs, data included in Fig. 2.

§ ^{26}Mg errors are observed variations between individual analyses of the standard.

$^{26}\text{Mg}/^{24}\text{Mg}$ can be seen to correlate strongly with $^{27}\text{Al}/^{24}\text{Mg}$; the maximum ^{26}Mg excess of $1,064 \pm 27\%$ (2σ) at $^{27}\text{Al}/^{24}\text{Mg} = 17,200 \pm 1,700$ is the highest so far recorded. However, the initial $^{26}\text{Al}/^{27}\text{Al}$ ratio of $8.4 \pm 0.5 \times 10^{-6}$, given in Fig. 2, is much less than the commonly observed value of 4.5×10^{-5} recorded for carbonaceous chondrite CAIs^{9,10,14,22-25}. $^{25}\text{Mg}/^{24}\text{Mg}$ ratios do not correlate with $^{27}\text{Al}/^{24}\text{Mg}$ as would be expected if the apparent variation in Mg content were due to primary beam overlap onto the olivine-bearing matrix. Overlap onto the included spinel can similarly be excluded as the Mg in this phase is isotopically normal (Table 2); the hibonite would therefore appear to be zoned with respect to Mg.

The HAL hibonite-rich inclusion has been extensively studied^{20,21} and it has been shown that while the oxygen and calcium isotopes of the hibonite are anomalous (fractionated by 30% and 7.5% per mass unit respectively), the measured magnesium isotopic composition is within error of the terrestrial standards²⁶. The $^{25}\text{Mg}/^{24}\text{Mg}$ ratio measured by ion microprobe (Table 2) is well within the $\pm 5\%$ limit set by the reproducibility of the terrestrial hibonite standard and no $\delta^{26}\text{Mg}$ excess is observed. The 2σ error limit for $\delta^{26}\text{Mg}$ gives an upper limit, for the initial $^{26}\text{Al}/^{27}\text{Al}$ ratio, of 9×10^{-8} for this inclusion and a 5% per mass unit limit for any mass fractionation. The HAL hibonite grain analysed was zoned with respect to Mg, the greater part of the grain having an $^{27}\text{Al}/^{24}\text{Mg}$ ratio in excess of 3,300. The Mg contents are somewhat lower than was found for the whole crystal analyses ($^{27}\text{Al}/^{24}\text{Mg}$ ratio $\sim 2,400$ after blank correction²⁶); this may be explained by the variability of the Mg content.

The high-temperature minerals in the Al-rich chondrules from ordinary chondrites crystallized from a melt and are not direct equilibrium condensates¹. It is possible, therefore, that ^{26}Al was present within precursor Ca-Al-rich solids but had decayed before melting (and isotopic homogenization) occurred.

Certainly *in situ* isotopic homogenization, after accretion, is unlikely for ALHA 77299 since the plagioclase within the chondrule is not of uniform chemical composition and the hydrogen isotopic composition of the meteorite's matrix is enriched in deuterium²⁷. Assuming an initial ubiquitous presence of ^{26}Al , with an initial $^{26}\text{Al}/^{27}\text{Al}$ ratio of 4.5×10^{-5} , the ^{26}Mg error limits combined with the $^{27}\text{Al}/^{24}\text{Mg}$ ratio indicate that the ALHA 77299 chondrule crystallized (and accretion occurred) at least 4.5 Myr after the bulk of the carbonaceous chondrite CAIs were formed. In contrast, small ^{26}Mg excesses (initial $^{26}\text{Al}/^{27}\text{Al}$ ratio of 6×10^{-6}) have been found in an igneous-textured inclusion from Allende¹⁴ defining a possible crystallization age of only 2 Myr. The observed variations in $^{26}\text{Al}/^{27}\text{Al}$ may, however, simply reflect variations within the source material^{14,28}.

While no ^{26}Mg excesses were recorded for the plagioclase grains, the presence of excesses within the Dhajala hibonite, combined with the fact that they correlate with the $^{27}\text{Al}/^{24}\text{Mg}$ ratio, indicates that ^{26}Al was present when the fragment of one ordinary chondrite was formed. Taking the simplest view of an initially uniform $^{26}\text{Al}/^{27}\text{Al}$ ratio of 4.5×10^{-5} and that no Mg mobility has occurred, the Dhajala hibonite formed ~ 2 Myr after the majority of the carbonaceous chondrite CAIs. If it is assumed that a large body, with an average composition similar to that of Dhajala²⁹, has a $^{26}\text{Al}/^{27}\text{Al}$ ratio of 8.4×10^{-6} it can be shown (using the equations of Schramm *et al.*¹⁵) that the temperature increase within the core of the body would be less than 750 °C and melting would not occur. Further, while a common source for both the ordinary and carbonaceous chondrite Ca-Al-rich objects is possible, the Dhajala hibonite is chemically like the unusual HAL hibonite from Allende. The source of these hibonite-bearing inclusions may differ from that of the majority of the Ca-Al-rich inclusions within chondrites. Therefore, while ^{26}Al is present within the fragment of one

ordinary chondrite, it cannot necessarily be inferred to be present throughout either Dhajala itself or the ordinary chondrites as a whole. ^{26}Al presence has been extended but its importance as a widespread heat source in condensed Solar System bodies is still in doubt.

We thank B. Mason, R. S. Clarke Jr, W. Zeitschel, E. Olsen, I. D. Hutcheon and the Meteorite Working Group for the loan of specimens and thin sections. We also thank A. M. Davis for help in the use of the JEOL SEM. This study was supported in part by NASA grants NGL 32-004-064 and NAG-9-30 to K. Keil (A.B.), grants NGL 14-001-169 and NAG 9-51 to R. N. Clayton (R.W.H.), and by a Deutsche Forschungsgemeinschaft grant to D. Stöfler (A.B.).

Received 11 August; accepted 20 December 1983.

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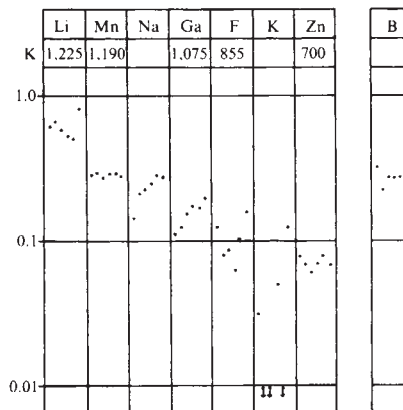


Fig. 1 C1 chondrite and silicon normalized data for moderately volatile elements. Data are listed in the order (left to right): D-1, Ka-168, KH-1, Po-1, Fr-1, SC-1. Boron data from this study, all other data from ref. 1. Condensation temperatures (K) are shown for some elements¹⁰.

method applicable at low boron abundances. We have recently constructed an analytical instrument that satisfies these needs³. Prompt γ neutron activation analysis (PGNAA) which has been used sporadically over the past dozen years (for example, ref. 4) permits measurement of γ -rays emitted during neutron irradiation of samples. Our procedure is as follows. Samples weighing approximately 1 g are irradiated external to the reactor core in a flux of $6 \times 10^7 \text{ n cm}^{-2} \text{ s}^{-1}$; γ rays are counted by a 14% efficient intrinsic germanium detector 25 cm from the sample. The boron blank of the system has been carefully measured and found to be independent of sample size and composition. The small sodium interference on the boron peak was compensated using the method of Curtis *et al.*⁴. Contamination problems are minimized as sample preparation is limited to crushing and packaging. We have calibrated our system using chemical standards and find good agreement with values of the United States National Bureau of Standards and other standards (Table 1). At the abundance levels encountered in this study the main limitation of the accuracy is in the measurement of the blank of the system. The blank is contributed by boron-bearing detector components close to the sample position, and hence is very stable. The detection limit is 0.1 p.p.m. and precision is $\pm 25\%$ at the abundance levels encountered in this study.

The purpose of the present study is to interpret the behaviour of boron during the condensation of the solar nebula and the subsequent evolution of the planetary system. Information derived both from meteorites and from terrestrial materials is necessary for this to be done, and the emphasis during the analytical programme has been placed on the latter.

Spinel lherzolite xenoliths from alkali basalts are believed to be derived from the upper mantle. However, the upper mantle is known to be heterogeneous and the xenoliths exhibit various stages of depletion (extraction of magma) and enrichment (metasomatism). The samples used in this study were supplied by E. Jagoutz (Max-Planck-Institut für Chemie, Mainz) and are from flows in France, Germany, Austria and the USA. These samples were selected on the basis of mineralogical and textural criteria to represent fresh, uncontaminated fertile mantle¹.

It is difficult to determine whether this objective has been achieved. However, the consistency of many elemental abundances from the diverse localities suggests that the samples are sufficiently representative for the purposes of this report. In addition the problem is reduced because we consider the relative abundance of different elements in the samples, thus the results are not affected by cosmochemical processes that raise or lower the abundances of all the elements of interest. The effects of extensive geochemical perturbations would be readily recogniz-

Boron cosmochemistry interpreted from abundances in mantle xenoliths

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Recent technical advances have made it possible to measure the abundance of boron in ultramafic xenoliths from alkali basalts thought to be representative of the upper mantle. Such data can provide information on the cosmochemistry of boron, particularly the temperature and mode of condensation of this element from the solar nebula. Analysis of xenoliths, selected by Jagoutz *et al.*¹ to represent fertile unaltered mantle, shows that the mean boron abundance is more than three times that expected for the condensation temperature of 700 K calculated by Cameron *et al.*². Our measurements, reported here, suggest that boron condensed at approximately 1,200 K as a solid solution in alkali feldspar or anorthite and not as an independent phase.

Boron geochemistry has been neglected, except in sediments, mainly due to lack of a rapid and accurate instrumental analytical

Table 1 Boron in international geochemical reference standards

Standard		B (p.p.m.)	
		Measured [†]	Other estimates
NBS 1571	Orchard leaves	32	33 *
NBS 1633a	Fly ash	41	39 †
USGS PCC1	Peridotite	1.4	1.4 †
USGS DTS1	Dunite	0.5	—

* Certified value.

† Ref. 13.

‡ Ref. 14.

able, as they would destroy the correlation between condensation temperature and abundance (see later).

The data (Table 2) have a mean of 0.53 ± 0.07 p.p.m. (1σ) or 6.5 ± 0.8 atoms B per 10^6 atoms Si (ref. 1). The uncertainty is comparable with the accuracy of the method at these abundance levels, hence there are no statistically valid differences between samples. Two items of data suggest that this value is representative of unfractionated mantle material: (1) the abundance is consistent with pyrolite models of the mantle, if we assume no boron in dunite and 3 p.p.m. boron in mid-ocean ridge basalts⁵; (2) it is higher than analyses of other unmetasomatized spinel lherzolite xenoliths, where lower Al abundances indicate that magma has been extracted⁶.

In order to interpret these data we must next consider the cosmic (or solar system) abundance of boron and other moderately volatile elements, as derived from meteorite data. Cosmic abundances are well known for most elements but not for boron^{7,8}. The arguments that follow are critically dependent on the cosmic abundance chosen, hence the subject will be discussed in detail. Earlier estimates take no account of the class of meteorite from which the data were derived. For instance Curtis *et al.*⁷ in their study of meteorites obtained a boron abundance of $6.6^{+6.5}_{-3.3}$ atoms B per 10^6 atoms Si. However, boron is a moderately volatile element² and hence is expected to occur only in the meteorite matrix and not in chondrules. Anders and Ebihara⁸ applied a correction, for the dilution effect of chondrules, to the data of Curtis⁷ and obtained a boron abundance of 24 ± 5 atoms B per 10^6 atoms Si. The lower uncertainty in this value gives it greater weight, compared with the uncorrected data of Curtis⁷. However, Curtis's value agrees better with cosmic abundance estimates derived from spectroscopic observations of the Sun⁹, and hence should not be totally dismissed. The matter may be resolved when analyses of separated chondrites and matrix become available.

Abundances of both boron and the moderately volatile elements determined by Jagoutz *et al.*¹ have been normalized relative to Si and C1 chondritic abundances, following Anders and Ebihara⁸ in the case of boron; the results are shown in Fig. 1. The mean boron depletion value of the samples relative to chondrites is 0.27 in units of B per 10^6 Si atoms, which is in the range of the other moderately volatile elements. Normalization using the cosmic abundance of Curtis⁷ would give a depletion value of 0.98, which is similar to the refractory elements (for example, Al, Ca, La), and its implications will be considered shortly.

Table 2 Abundances of boron in ultramafic xenoliths

Sample	B (p.p.m.)
D-1	0.64
Ka-168	0.44
KH-1	0.53
Po-1	0.55
Fr-1	0.55
SC-1	0.46
Mean	$0.53 \pm 0.07^*$

The xenoliths were selected by Jagoutz *et al.*¹ to represent fresh unaltered mantle.

* ± 1 s.d.

The moderately volatile elements have been arranged in Fig. 1 in order of decreasing nebular condensation temperatures¹⁰. Jagoutz *et al.*¹ pointed out the good correlation between depletion values and condensation temperatures, which thus allows us to estimate the temperature of condensation of boron as approximately 1,200 K. This temperature is in contrast to the calculations of Cameron *et al.*² which suggested a condensation temperature of 700 K, similar to that of Zn (Fig. 1). Such a low temperature would imply a depletion value for boron of ~ 0.08 , which would correspond to an abundance of 0.16 p.p.m. Our value of 0.5 p.p.m. boron in the xenoliths is clearly much greater than this, even after allowing for analytical error and uncertainties in the chondritic abundance. We do not believe that boron could have been introduced by contamination during preparation. Therefore, we consider that the calculations of Cameron *et al.*² are in error.

Their calculations were based on the following assumptions: (1) that the compounds considered (mostly borides and borates) are the most refractory crystalline compounds of boron; (2) that boron did not condense as a solid solution in other phases at higher temperatures. It is unlikely that there exist refractory boron-bearing phases not considered by Cameron *et al.*² that could condense at such drastically higher temperatures. Therefore, it is most likely that the second assumption of Cameron *et al.*² is in error and that boron condensed in solid solution in high temperature phases.

Substitution of boron into crystalline structures is limited by its high charge density. However, it is known to substitute for aluminium in some minerals in terrestrial conditions. Refractory aluminium phases include corundum, hibonite, melilite, anorthite and alkali-feldspar¹¹. Substitution into a feldspar is most likely because these minerals condense at approximately 1,200 K and such substitution under terrestrial conditions is well known¹².

In the event that the chondritic abundance of Curtis⁷ proved correct, our data would indicate that boron condenses with the refractory elements. This could be achieved by solid solution in corundum, hibonite or melilite. However, there is no evidence that such substitutions do occur. Further research is obviously needed here.

The most acceptable interpretation of our data is that boron condenses from the solar nebula at approximately 1,200 K in solid solution in one of the feldspars. It does not condense with the most refractory phases or as a separate phase.

Received 25 August; accepted 7 December 1983.

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The iron–water reaction and the evolution of the Earth

Yuh Fukai

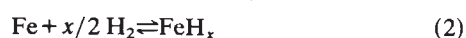
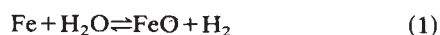
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The possibility of dissolution of hydrogen in the Earth's core through the reaction $\text{Fe} + \text{H}_2\text{O} \rightarrow \text{FeO} + \text{FeH}_x$ was noted by Stevenson¹, but has since been largely disregarded because the solubility of H in Fe was thought to be too low and the volatility of H_2O too high to deserve serious consideration². However, a recent paper³ from this laboratory showed that the solubility of H in Fe is very large at high pressures, and that H could indeed be a major cause of the density deficit of the Earth's outer core. In this paper I demonstrate, by investigating the nature of the iron–water reaction in more detail in light of recent advances in the knowledge of impact dehydration of planetesimals in the accretion process, that the iron–water reaction may well have played a decisive role in the evolution of the Earth.

The scenario for evolution used here presumes that primordial material consisted of a 9:1 mixture of high-temperature and low-temperature condensates similar to E- and CI-chondrite, respectively. This hypothesis, originally invoked to account for the abundance of volatile elements in the Earth^{4,5}, has found additional support in recent years: infrared spectrometry has shown that asteroids in the main belt are classified into two distinct types similar in reflectance to E- and C-chondrite⁶, and oxygen isotope analysis has shown that, among various planetary materials, only E- and CI-chondrites fall on the terrestrial line of fractionation^{7,8} and are therefore accessible as primordial material.

More crucial is the assumption that a substantial part of the water, supplied as structural water in serpentine and/or chlorite in the low-temperature component, was retained in the growing Earth. According to Safronov⁹ and Weidenschilling¹⁰, the accretion heating was rather small; for accretion times of $\sim 10^7$ – 10^8 years, the surface temperature of the growing Earth did not exceed ~ 350 K, which is much lower than the dehydration temperature of the hydrous silicates (≥ 900 K). Based on this and known dehydration characteristics of serpentine, Lange¹¹ has evaluated the loss of water in the accretion process. He found that the partition of water inside and outside the Earth depends critically on the dehydration efficiency (DE = water leaving solid planet and released to atmosphere ÷ potential water loss due to shock heating in a single accretional event) and in particular, that all water can be retained in the Earth if DE is smaller than $\sim 1/4$. Although no reliable estimates of DE are available, these results strongly suggest that a major part of the water could indeed be retained in the proto-Earth.

The reaction that takes place in the growing Earth when H_2O in the low-temperature component (C) comes in contact with Fe in the high-temperature component (S) can be divided into two steps:



Thermodynamical calculations show that reaction (1) proceeds to the right under all conditions of pressure (P) and temperature (T) encountered in the interior of both the present Earth, and probably also of the growing Earth in the later stages of accretion. Thus all water in the growing Earth will probably have reacted with Fe except in the surface region where the temperature was not high enough to decompose the structural water into molecular form. The solubility of H in Fe (equation 2) was calculated using the heat of solution at normal pressure

Table 1 Composition of the primordial material (wt%)

	C-component*	S-component†
Fe	0	34.8
Ni	0	1.9
FeS	5.65	0
SiO ₂	21.74	39.4
Al ₂ O ₃	1.59	1.8
FeO	22.86	0
MgO	15.24	20.7
CaO	1.18	1.3
H ₂ O	19.19	0
C	2.99	0

* Orgueil (CI-chondrite). The value for FeS is given as S.

† Volatiles (including S and C) have been removed from the composition of St Marks (E-chondrite)⁵.

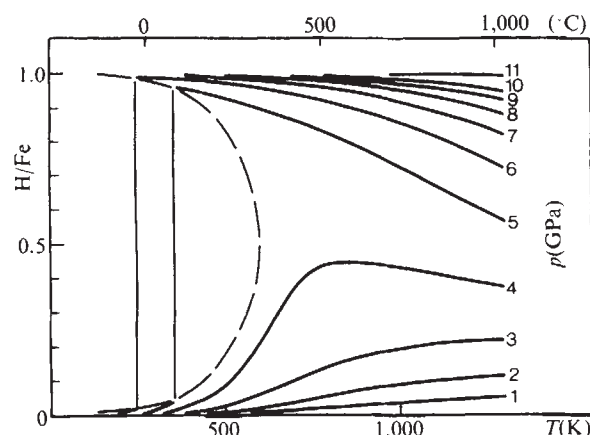


Fig. 1 Solubility of H in Fe calculated as a function of p , T . Two-phase separation occurs as a result of elastic interaction between H atoms¹².

and the equation of state of fluid H_2 at high pressures¹². The maximum concentration was assumed to be $[\text{H}]/[\text{Fe}] = 1$ by analogy with other metal–hydrogen systems, and the approximate elastic interaction between H atoms was included. The solubility thus obtained is shown in Fig. 1, as a function of p , T . The result is in good agreement with experimental data reported at a few selected values of p , T (refs 12–14). The overall results show that the reaction



occurs completely at $p > 4$ GPa and $T > 700$ K. The pressure of 4 GPa corresponds to a depth of 110 km below the surface, where the temperature is expected to be higher than 1,000 K. For the quantitative discussions below $\delta = 80$ km is taken as a boundary between the inner region of complete occurrence of reaction (3) and the surface region where reaction (3) can be disregarded.

It should be noted that the melting point of Fe is reduced appreciably by dissolution of H: solidified droplets of iron can be observed after reaction of Fe, $\text{Mg}(\text{OH})_2$, MgO and $\text{SiO}_2 \cdot x\text{H}_2\text{O}$ at 1,200 °C and 5.4 GPa¹⁵, implying that the melting point of Fe is lowered by at least 500 K.

The scenario for the evolution of the Earth which these data support is as follows. From the early stages and throughout the accretion process of the Earth, the $\text{Fe}-\text{H}_2\text{O}$ reaction occurs continuously in the inner region of the growing Earth, and molten blobs of the reaction product FeH_x sink to form a proto-core. Some other elements, such as O and S, may also be dissolved on the way. Subsequently, the surface layer, being heavier than the Fe-depleted proto-mantle, is bound to undergo convective overturn. During this process, the reaction of Fe

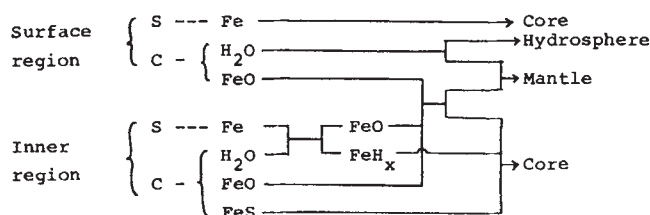


Fig. 2 Mass-flow diagram of the evolutionary process of the Earth.

(contained in blocks of S-component) with water (in blocks of C-component) is not expected to occur as effectively. Thus, the Fe sinks to join the proto-core without dissolving any appreciable amounts of hydrogen, and the water is largely degassed to form a hydrosphere.

To make the model more quantitative, I adopt chemical compositions of C- and S-components listed in Table 1, and compute the materials budget according to the mass-flow diagram shown in Fig. 2. For simplicity of calculation, the Ni content in the S-component was included with Fe.

The amount of FeO dissolved in the core can be calculated from the constraint that the amount of FeO in the mantle is 5.0%, which derives from the ratio $\text{FeO}/(\text{FeO} + \text{MgO}) = 0.12$ in the mantle and the average MgO content of 20.6%. The sum of the materials that enter the core amounts to 33.2%, close to the value of 32.4% obtained from a recent seismological analysis (PREM)¹⁶. As the amount of Fe coming from the surface layer is nearly equal to that of the inner core, it can be conveniently disregarded in calculating the composition of the outer core. The result obtained is $\text{FeH}_{0.36}\text{O}_{0.12}\text{S}_{0.03}$. (The Fe from the surface layer was presumably the origin of the inner core.)

The density reduction to be caused by dissolution of these light elements can be estimated from

$$-\frac{\Delta\rho}{\rho} = \frac{0.19x}{1+0.19x} + \frac{0.36y}{1+0.65y} + \frac{0.71z}{1+1.28z} \quad (4)$$

which applies to $\text{FeH}_x\text{O}_y\text{S}_z$ at the core-mantle boundary. This expression has been derived from shock-compression data on FeO, FeS systems¹⁷, and theoretical calculations on the Fe-H system (K. Terakura, personal communication). The density reduction corresponding to the outer-core composition given above is $-\Delta\rho/\rho = 0.064(\text{H}) + 0.040(\text{O}) + 0.020(\text{S}) = 0.124$, in reasonable agreement with the 'observed' value, 0.092 ± 0.02 , obtained from comparison of PREM¹⁶ and the density of liquid Fe under equivalent conditions of p, T (ref. 18).

Conversely, the amount of water incorporated in the proto-Earth so as to reproduce the 'observed' density reduction of the outer core can also be estimated. This leads to the conclusion that about 25% of the water in the C-component was lost in the accretion process (a reasonable value in view of Lange's estimates¹¹), and the composition of the outer core is $\text{FeH}_{0.27}\text{O}_{0.07}\text{S}_{0.03}$. More detailed discussions on the core composition may not be warranted without better knowledge of the composition of the primordial material. (Retention of sulphur in the S-component, for example, tends to reduce the necessary amount of H.)

Finally, the evolutionary scheme presented here also has profound implications for the formation of atmosphere/hydrosphere, since it implies that Fe served as a scavenger of volatile elements H, C, N in the interior of the growing Earth and brought them into the core. The intact surface layer remaining would then have degassed during convective overturn to produce the atmosphere/hydrosphere. The convective overturn, which took place shortly after accretion had been completed, provides a natural explanation for the 'catastrophic degassing', which, as shown by Ar-isotope studies, occurred in the very early history of the Earth¹⁹.

I am indebted to Professors S. Akimoto, Y. Syono, and N. Onuma for helpful discussions, and to H. Sugimoto and T. Ohtani for assistance in thermodynamical calculations.

Received 11 August; accepted 7 December 1983.

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Determination of oxygen functionalities in synthetic fuels by NMR of naturally abundant ¹⁷O

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Heteroatomic (N, S, O) species are a significant factor in the stability and usefulness of natural and synthetic fossil fuels. Although sulphur and nitrogen removal have received much attention, oxygen is also important, being the most abundant heteroatom in most coals and requiring consumption for its removal, a requirement which can adversely affect the economics of coal liquefaction. Oxygen bonds (for example, ethers), on the other hand, may be the 'weak link' in the depolymerization of coal. Also, oxygen functionalities may interfere with the desulphurization and denitrogenation processes. Thus, the unequivocal identification and quantitative determination of oxygen functionalities could greatly aid in the design of processes for producing and upgrading synthetic fuels in a cost-effective manner^{1,2}. We now report the successful use of nuclear magnetic resonance (NMR) of naturally abundant ¹⁷O to determine oxygen functionalities in synthetic fuels.

¹⁷O-NMR has found only limited use in the study of natural substances because of its low natural abundance (0.037%) and the broad lines encountered due to its quadrupolar nucleus ($I = 5/2$). However, with improvements in Fourier transform NMR instrument sensitivity, ¹⁷O-NMR may become very important as a tool for studying oxygen functionalities in natural systems previously considered to be too low in oxygen for successful study³. Despite its difficulties, ¹⁷O-NMR has several important advantages. The chemical shift of ¹⁷O has a wide range of ~800 p.p.m., and the ¹⁷O chemical shifts of many small (molecular weight <100) compounds have been reported throughout this range⁴⁻⁸. The quadrupolar nature of the nuclei which broadens the observed lines also shortens the spin-lattice relaxation time (T_1) of the nuclei, such that very rapid pulsing is possible; more than a million transients can be recorded

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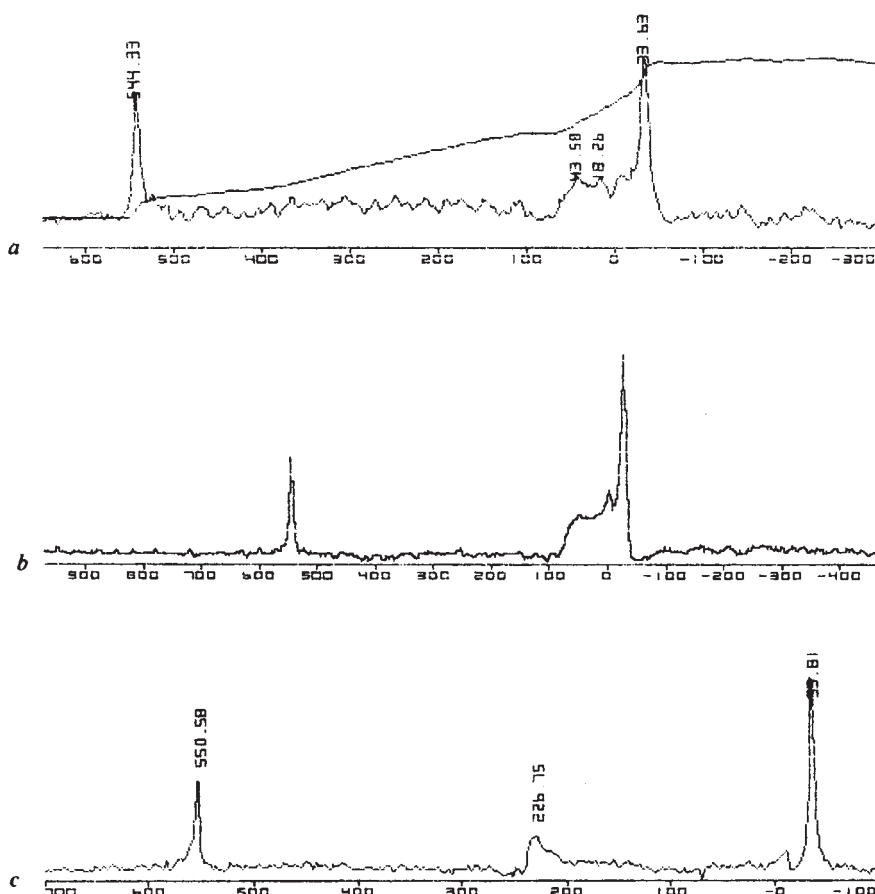


Fig. 1 ^{17}O -NMR spectra of coal liquids. *a*, Very weak acids (A1); *b*, weak acids (A2); *c*, strong acids (A3). All chemical shifts are referred to water.

overnight. Determination of the relative amounts of the various oxygen functionalities can also be done, if ^{17}O spectra with sufficient signal-to-noise ratio are available and the various signals are resolved.

The samples used in our study were the heavy distillates produced from Powhatan No. 5 coal using the SRC-II process⁹, and three acidic and two basic fractions that had been prepared chromatographically from the heavy distillate¹⁰. The oxygen content of the samples was total heavy distillate, 2.3%; very weak bases, 7.1%; strong bases, 1.8%; very weak acids, 8.9%; weak acids, 9.8%; and strong acids, 17.9% (see ref. 10 for additional data on these materials).

The present data were obtained with a Varian XL-200 spectrometer using a 14- μs pulse (90°), a 13-ms acquisition time and a 20-ms delay. Using this sequence, about 30 transients s^{-1} or 108,000 h^{-1} can be obtained, yielding a good spectrum within a few hours. Deuterated methylene chloride was used as the solvent for acid and base fractions; it was also used in all samples as the field frequency lock solvent. From 3×10^5 to 2.7×10^6 transients were recorded before Fourier transformation. Figure 1 gives the ^{17}O spectra of the very weak acids (A1), weak acids (A2) and strong acids (A3). All three of these samples were spiked with 50 μl each of methanol and acetone to aid in the adjustment of the spectral phasing. Methanol appears as the prominent peak at ~ -35 p.p.m. and acetone is the prominent peak at ~ 540 p.p.m. All three spectra also exhibit a peak near 0 p.p.m., identifiable as water. Both the weak acid (A2) and the very weak acid (A1) spectra display a large broad peak, resolvable in A1, in the -10 to 80 p.p.m. region. This is characteristic of alcohols, phenols and ethers^{4,5}.

There seem to be some aldehyde and/or ketone functional groups in the very weak acid (Fig. 1*a*), as indicated by an upfield shoulder on the acetone peak. Both the very weak and weak acids as well as their hydrotreated products have been exhaustively studied by gas chromatography/mass spectrometry and gas chromatography methods (refs 11, 12 and L.P., D.W.G.

and B.C.G., in preparation). These methods show the very weak acid to contain dimethyl benzofuranone, cyclohexylphenol isomers, ethylphenoxybenzene, methylcyclohexylphenol, trimethyldihydronaphthalene, tetrahydronaphthol and phenylphenol. The weak acid contains tetrahydronaphthol, phenylphenol, methyltetrahydronaphthol, methylphenylphenol, cyclohexylphenols and dimethylphenoxybenzene. The ^{17}O spectrum of the strong acids (A3) shows only the prominent acetone and methanol peaks, a small peak around 0 p.p.m., probably due to water, and a peak at ~ 230 p.p.m. This is the appropriate spectral region for furans and the carbonyl oxygen of carboxylic acids. The chemistry of the separation procedure and the IR data indicate that carboxylic acids are the most likely candidates¹⁰.

The ^{17}O -NMR spectrum of the total heavy distillate was also recorded. Although 2.7×10^6 scans were used, the spectrum is noisy. Integration measurements indicate that about 19% of the oxygen is in the alcohol, phenol or ether region of -50 to 100 p.p.m. Phenols are most likely. About 44% of the oxygen is in the carboxylic acid ($\text{C}=\text{O}$) and furan region at 150 – 280 p.p.m. The chemical shift range of 310 – 380 p.p.m. contains $\sim 14\%$ of the oxygen. Esters are the only reported compounds having ^{17}O -NMR signals in this region^{4,5}; however, the ^{17}O -NMR shifts of many of the oxygen-containing aromatic compounds that may be found in coal liquids, such as quinones and aromatic acids, are unknown. The carbonyl region, due to ketones and aldehydes (480 – 550 p.p.m.), contains about 23% of the oxygen^{4,7}. These determinations are speculative at this stage and better spectra are needed for more reliable quantitative measurements.

As Fig. 1*a* shows, a very favourable situation for quantitative analysis exists in the ^{17}O spectrum of the very weak acid (A1). From the spectrum integration, about 4% of the oxygen is in the ketone or aldehyde region of 500 – 550 p.p.m., 29% is in the phenol, alcohol and ether region of -10 to 80 p.p.m. and the remainder is in the 100 – 500 p.p.m. region. If a sufficient

catalogue of ^{17}O chemical shifts of coal-like compounds existed, one might be able to split this region into oxygen functionality sections. Overall, the integration values for the coal liquid signals and the methanol and acetone signals are consistent with the 50 μl of each pure compound and a 1-g sample of coal liquid acid containing about 9% oxygen by weight.

The ^{17}O spectra of the sample of very weak bases (B1) and that of the strong bases (B3) have peaks at 0 p.p.m. in B3 or slightly negative in B1, due to traces of water. A strong peak from 30 to 80 p.p.m. in B1 is assigned to alcohols, ethers, phenols and higher aromatic alcohols. Phenol is reported to have a chemical shift of 69 or 79 p.p.m.⁴⁻⁶. The large peak in B3 at 250 p.p.m., also found in B1 as a smaller peak, may be due to furan-type oxygen. Furan is reported to have an ^{17}O chemical shift of 241 p.p.m.^{4,5}. The spectra of the strong bases (B3) lack any prominent ^{17}O peaks above 400 p.p.m. The strong resonance between 450 and 520 in B1 may be due to compounds such as acetophenones substituted by $-\text{OH}$ or NH_2 groups⁷. The presence of phenolic amines in this fraction has been indicated by other spectroscopic techniques¹³.

The IR spectra of A1 and A2, the very weak and the weak acids, and B1, the very weak base, are essentially identical. They consist mainly of a strong absorption centred at 3,400 cm^{-1} that is assigned to OH groups, a band between 2,800 and 3,050 cm^{-1} due to aromatic and aliphatic hydrogen, a very weak band at 1,700 cm^{-1} in the acids that may be due to $\text{C}=\text{O}$, bands at 1,600 and 1,400 cm^{-1} , also due to $\text{C}-\text{H}$, a strong band centred at 1,200 cm^{-1} due to OH and bands at 800 cm^{-1} , again due to aromatic $\text{C}-\text{H}$. Hydroxyl groups appear to be the only major non-hydrocarbon bands in the IR spectrum. In the ^{17}O -NMR spectrum, hydroxyl groups are the major peaks in A1 and A2, although some carbonyl oxygen also appears in A1. Some hydroxyl oxygen is present in the very weak base, B1, but there are also some other peaks, such as those in the furan region, that are not observed in the IR spectrum of this fraction. The strong base, B3, has the same CH bands as the others (A1, A2, B1) but lacks the strong hydroxyl bands at 3,400 and 1,200 cm^{-1} . There are some weak absorbances at 3,200–3,500 cm^{-1} which may be due to amines or a low relative concentration of hydroxyl groups. By ^{17}O -NMR, as discussed above, there is evidence of much more oxygen functionality in B3. There is good evidence of acids and other hydroxyl functionality in the IR spectra of the strong acid, A3, in agreement with findings in the ^{17}O spectrum.

With materials of low oxygen content ($\sim 1\%$), as much as 16 h of spectrometer time may be necessary to obtain useful spectra, incurring an expense that may discourage ^{17}O -NMR analysis of large numbers of routine samples. Since ^{17}O -NMR is specific to one atom and the signal area is directly proportional to the concentration of the functional group, it offers an excellent method of quantitative analysis. Thus, ^{17}O -NMR can be a very powerful tool for the investigation of oxygen functionalities in the complex mixtures present in synthetic fuels.

We acknowledge the support of the US Department of Energy for the preparation of the samples and DOE Contract DE-AC22-F90ET14880.

Petroleum generation by laboratory-scale pyrolysis over six years simulating conditions in a subsiding basin

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Many researchers have attempted to duplicate under laboratory conditions the geochemical reactions that lead to economic deposits of liquid and gaseous hydrocarbons¹⁻⁴. Experiments lasting from a few^{5,6} to several hundred⁷ days, usually at constant temperatures, have been devised. The differences in time scale and other parameters between geological processes and laboratory experiments are so great that the relevance of laboratory results is often questioned^{8,9}. Consequently, we have heated potential source material from 100 to 400 °C over six years, increasing the temperature by 1 °C per week. This was done in an attempt to simulate the thermal history of a sample being buried in a continuously subsiding basin with a constant geothermal gradient. After four years, a product indistinguishable from a paraffinic crude oil was generated from a torbanite, while a brown coal gave a product distribution that could be related to a wet natural gas. Of great significance is the absence of olefins and carbon monoxide in all products. We believe the present experiments, which are possibly as slow as can be realistically planned within a human time scale, have for the first time successfully duplicated hydrocarbon generation in a continuously subsiding sedimentary basin.

Two extreme types of source rock were chosen for this study: a Permian torbanite from Glen Davis (New South Wales, Australia) and a Tertiary brown coal from Loy Yang (Victoria). Each was extracted with chloroform and demineralized with HCl and HF to give a kerogen containing less than 0.3% mineral matter. Insoluble organic material was used so that the initial solid state decompositions would be studied, eliminating both catalytic effects of minerals and, in the case of brown coal, reactions of soluble natural waxes. A series of 1-g samples in open glass tubes was dried under nitrogen at 105 °C and each sample individually sealed in a separate stainless steel 'bomb' under nitrogen at atmospheric pressure. The bombs were placed in an oven at 100 °C and the temperature increased by 1 °C each week. Bombs were taken out periodically and cooled, and the mixture of nitrogen and generated gases was released and analysed. The residue in the glass tube was extracted with *n*-hexane and then with chloroform. The insoluble solid residue was dried under nitrogen. The hexane extract was analysed by gas chromatography-mass spectrometry (GC-MS) both before and after evaporation of the solvent under nitrogen.

Mass balance data for samples removed at yearly intervals are given in Table 1. Some tubes (particularly after six years) were difficult to open, leading to less precise results. The small yields of oil and asphaltenes after one, two and three years probably represent indigenous material not completely removed by chloroform extraction, which was only carried out before HCl and HF demineralization. For the brown coal, the last column in Table 1 probably represents mainly water, both retained after drying at 105 °C and generated during pyrolysis. For most torbanite samples, values in the last column are believed to be dominated by losses of powdery, solid material. An exception is the sticky torbanite residue after 200 weeks which was easily recovered, and the loss in this case is thought to be mainly light oil.

Probably the most interesting results were obtained from the samples heated to 300 °C over four years (Table 1). In particular, the torbanite data show that the main phase of oil generation has been entered during the fourth year of heating. After five years, cracking of oil had commenced and after six years only

Received 17 June; accepted 6 October 1983.

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Table 1 Slow pyrolysis of torbanite and brown coal for up to six years beginning at 100 °C

	Time (weeks)	Max. temp. (°C)	Product (% w/w of original, dry organic matter)				Losses, water and light oil (C ₆ -C ₁₀)
			Gas	Oil (hexane soluble, C ₁₁ +)	Asphaltenes (CHCl ₃ soluble)	Solid residue	
Torbanite	50	150	0.3	0.5	0.2	86.1	12.9
	100	200	~0.5	0.5	0.2	88.7	10.1
	150	250	0.7	0.7	0.2	85.3	13.1
	200	300	3.5	34.5	6.9	44.8	10.3
	250	350	42.3	3.1	0.1	34.5	20.0
	300	400	~45	0.0	0.0	~35	~20
Brown coal	50	150	1.4	0.4	2.3	90.9	5.0
	100	200	6.1	0.7	1.6	84.2	7.4
	150	250	16.1	0.2	1.2	78.1	4.4
	200	300	21.4	0.2	0.8	66.1	11.5
	250	350	~40	0.0	0.0	~50	~10
	300	400	~35	0.0	0.0	~55	~10

gases and residual solids (atomic H/C ~ 0.3) remained in both samples. Chromatograms of the hexane-soluble products obtained after four years are shown in Fig. 1. The compositions of the gases generated from the two source rocks after 200 weeks were as follows (all % by volume): Torbanite—CH₄ 32.1, C₂H₆ 16.7, C₃H₈ 13.0, *n*-C₄H₁₀ 4.8, *n*-C₅H₁₂ 0.1, C₆+ 2.3, CO₂ 31.0. Brown coal—CH₄ 4.7, C₂H₆ 3.9, C₃H₈ 4.2, *n*-C₄H₁₀ 0.7, *n*-C₅H₁₂ 0.1, C₆+ 0.2, CO₂ 86.2. Unlike rapid pyrolysis at higher temperatures¹⁰⁻¹⁴, olefins were completely absent from gaseous and oily products. Pyrolysis in the presence of water may result in decreased olefin formation but, significantly, the present experiments were carried out on virtually dry samples. Similarly carbon monoxide, a common product from coal and kerogen pyrolysis^{11,13,15}, was not detected. The absence of olefins and carbon monoxide has been noted in petroleum and natural gas from many parts of the world.

The torbanite kerogen produced ~40% *n*-alkanes with a typical paraffinic oil distribution from C₁₀ to C₃₀ (Fig. 1). Branched and cyclic compounds were minor components, pristane and phytane were not detected and there was virtually no

odd or even carbon preference. The major product from brown coal kerogen was carbon dioxide with only a minor amount of C₁₁+ oil. Significantly, this level of CO₂ was only slightly higher than that found in a bomb removed after three years (maximum temperature 250 °C). The torbanite 300 °C gas is similar to some natural gases but, at first sight, the gaseous hydrocarbon distribution from the brown coal is somewhat unexpected. However, by further heating, the unusually high relative content of C₃H₈ is removed and a hydrocarbon distribution consistent with many wet natural gases is obtained, for example the composition of the brown coal 350 °C gas is CH₄ 22.2, C₂H₆ 5.7, C₃H₈ 1.2, C₄H₁₀ 0.7, CO₂ 70.2 (all % by volume). Of the two source materials, at 300 °C the brown coal had generated a greater proportion of branched and cyclic alkanes, including pristane and phytane (Pr/Ph ~ 6), compared with normal alkanes.

Overall, the four-year results provide experimental proof of torbanite acting as an oil source and of brown coal being a source first of CO₂ and then of mainly gas/condensate. It should be remembered that the experiments relate to a situation which is possibly unusual in a geological context—one in which hydrocarbons do not migrate away from their source rocks as they are generated.

Other analyses, including a detailed petrographic study, are being carried out. A particularly important maturity parameter, vitrinite reflectance (*R*₀), is readily measured on the brown coal products and shows a smooth, but accelerating, increase with time: original 0.28%, 50 weeks 0.31%, 100 weeks 0.47%, 150 weeks 0.59%, 200 weeks 1.00%, 250 weeks 1.85%, 300 weeks 2.73%. However, the present experiments cannot be used to accurately define the oil generation window⁹.

Geological maturation events commonly involve 10³–10⁶ years or more at near maximum temperatures¹⁶; most laboratory pyrolysis studies are carried out on a time scale of 10⁻⁴ years. Many experiments using rapid or flash heating are of course designed to obtain structural information on geological materials and simulation of natural maturation is not an objective. The present experiments (10⁰–10¹ years) narrow the 'kinetic gap' and we believe are the best attempt so far to duplicate natural, subsiding, sedimentary conditions. Extensive conversion of kerogen to hydrocarbons has been achieved at less than 300 °C under noncatalytic conditions with a minimum of water present. A temperature of 250–300 °C is probably still much higher than is appropriate for substantial generation in many basins. However, it is considerably lower than the temperatures (~450 °C) required for significant C—C bond rupture on a time scale of minutes. The products of these slow 'molecule-by-molecule', solid-state decompositions are all typical of natural gases and petroleum, with no olefins or carbon monoxide being formed.

We thank T. D. Gilbert for assistance with GC-MS analyses and A. J. Bennett for reflectance measurements.

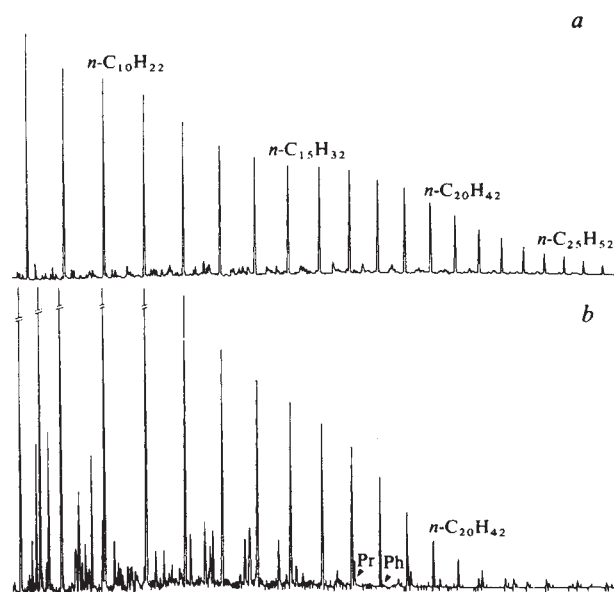


Fig. 1 Gas chromatograms of total hexane-soluble oil (before evaporation) formed after four years under nitrogen (initial temperature 100 °C, final temperature 300 °C, heating rate 1 °C per week). Oil from torbanite represents ~40% of original, dry, mineral-free kerogen (shown in *a*), and from brown coal <1% of original, dry, mineral-free kerogen (*b*). GC separation was achieved on a 50-m WCOT capillary column coated with OV101 programmed to 270 °C at 4 °C/min. Pr, pristane; Ph, phytane.

Received 26 September; accepted 20 December 1983.

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The gait of *Hipparion* sp. from fossil footprints in Laetoli, Tanzania

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Three *Hipparion* trails, two from adults and one from a juvenile dating from about 3.5 Myr ago, have now been found in Laetoli. Comparison of the *Hipparion* footprints with a variety of imprints made by domestic horses showed that the animals moved with a running walk over the soft and slippery soil of Laetoli: in the alternative hind–fore–hind–fore footfall sequence one foot is always close to the ground and can take over the body weight if the supporting foot slides. The smaller juvenile was taking relatively longer strides than the adult. Body support was provided mainly by the third main toe. The evidence indicates that volcanic ash was falling at the time the footprints were made, suggesting that the animals were passing through the area at about the same time. The criss-cross pattern in the trails of the juvenile and adult *Hipparion* can be seen in the travelling behaviour of mares and foals of living horses. Evidence that the *Hipparion* were moving with a running walk supports the view that this gait is not ‘man-taught’ for rider-comfort, but a natural ability in modern horses.

Apart from dinosaur trails¹, analyses of fossil footprint trails have hitherto been very limited except for Webb² who ascertained the gaits of fossil mammals by comparison with those of living animals. In 1979 trails of a number of fossil mammals, including hominids, were discovered by Dr Mary Leakey and her team at Laetoli, Tanzania³. The imprints were made about 3.5 Myr ago in soft lava, which was subsequently covered with volcanic ash (locality: Tuff 7, Site G). It is reasonable to suppose that the trails were all made at approximately the same time, and their excellent preservation offers a unique opportunity to study the locomotion of fossil mammals. Three trails (Fig. 1a–c) originated from a tridactyl equid. They run parallel to the hominid imprints, but travel in an opposite (north–south) direction (see Fig. 1).

The fossil bones found at Laetoli indicate that the equid concerned is a species of *Hipparion*. It was a rather sturdy animal about 1.00 m in body length⁴. The difference in size between the hoofs (that is, mean length of 6.5 cm in trails a and b and 3.5 cm in trail c) can be explained by an age difference between the animals involved with trail a and b originating from adult animals and c from a juvenile individual (see Fig. 1). Analysis of the fossil trails indicated that during locomotion *Hipparion* was supported mainly by the middle (III) toe. There is also evidence of side toe (II and IV) support in imprint 7 of trail b.

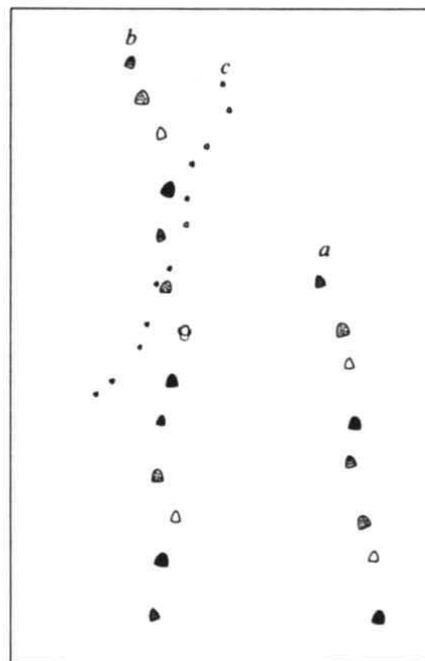


Fig. 1 Overview of three *Hipparion* trails found at Laetoli, Tanzania, 1979. Trails a and b, adults; trail c, juvenile. Trail a has a length of 251 cm and a maximum width of approximately 25 cm, the eight footprints are between 26 and 42 cm apart. Trail b is situated 25 m south of trail a. It is 497 cm long and has a maximum width of 30 cm; the footprints are between 30 and 35 cm apart. It is comprised of 13 imprints, of which the first 7 and the last 3 have clearly chiselled vertical rims, and the remaining 3 are somewhat larger and show slightly raised walls. Trail b curves east and is crossed by trail c between its 7th and 10th footprint. Trail c commences 130 cm and 35° south of trail b; the first six footprints are located east of trail b with the subsequent footprints situated west of b, turning east and running parallel to trail b. All 12 footprints in trail c have incompletely preserved vertical walls and a gentle inward slope. The trail is 251 cm long and 30 cm in maximum width. The distance between footprints is 17 cm and 31 cm. The above measurements were initially taken *in situ* by Dr Paul Sondaar, who also made the latex casts of trails a and b. The cast of b also contains footprints 6, 7, 8 and 9 of trail c where it crosses b. The casts were used for an analysis of the probable type of locomotion.

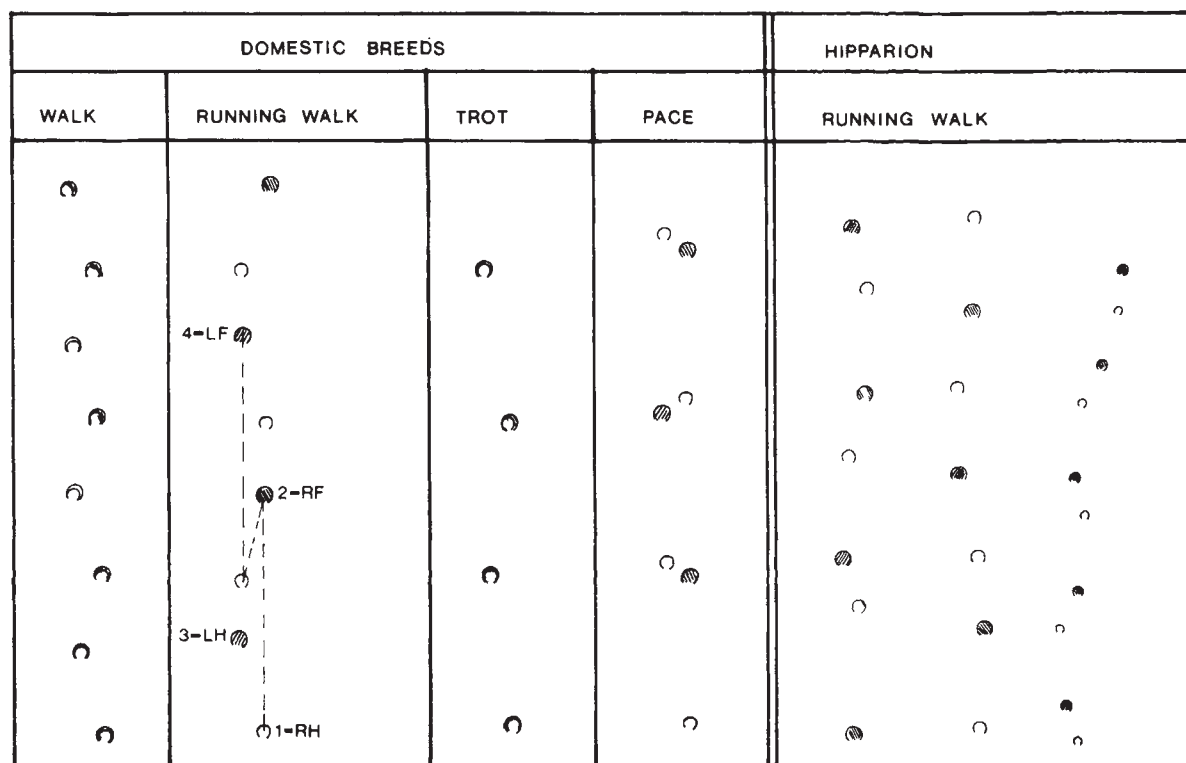


Fig. 2 Footprint patterns of domestic breeds compared with the *Hipparion* trails. The footfall sequence of the running walk is given by numbers 1, 2, 3 and 4. The right hind, RH; right front, RF; left hind, LH and left front; LF leg. The hind foot oversteps the fore foot by 25% of the stride length.

One may compare locomotion of *Hipparion* with that of recent equids, since, with the exception of the side toes, the general conformation and geometrical dimensions of the limbs and body of monodactyl and tridactyl equids are similar. This may be exemplified by an analysis of the ratios of hind-leg length and body length (0.83) and fore-leg length and body length (0.68) of mounted fossil and recent equid skeletons⁵⁻¹⁰.

In *Hipparion* and in recent horse the main hind hoof is narrower than the fore hoof⁵. Thus it is possible to differentiate between fore and hind imprints, to measure stride length and to decipher footfall sequences in fossil trails. Moreover, it is possible to determine both the type of gait of the animal (footfall sequence pattern) and from the trails of similarly sized recent equids the approximate speed at which the *Hipparion* was moving. In living horses the choice of gait depends primarily on the speed of movement¹¹ with the walk being chosen at slow speed, the trot at moderate speeds and the gallop at high speeds. However, certain breeds of horses may select alternative gaits at higher speeds. The pace (a gait where lateral pairs of legs are moved together) may be substituted for the trot (where diagonal pairs of legs are involved) and the 'running walk' (also known as the 'rack', 'amble' or 'tölt', depending on the breed concerned) may be substituted for the trot or pace. The running walk is the only gait the elephant can select for faster speed¹². The ability of particular horse breeds to select these alternative gaits has an anatomical basis (L. M. Slade, R. E. Montgomery & D. D. Rains, personal communication), the genetics of which are not fully understood¹³.

The *Hipparion* footprints were compared with imprints made by a variety of recent horses and it was found that the gait of *Hipparion* was similar (in footfall sequence) to the 'running walk' (see Fig. 2), which can be observed in Icelandic horses, Racking horses, Tennessee Walking horses, Peruvians and Pasco Finos. This gait has the same footfall sequence as the walk (right hind, right fore, left hind, left fore) but differs from it in speed and in duration of the periods of leg support. In the walk two or three feet are on the ground at any one time and the speed is comparatively slow. Stability requires that the centre of gravity

is either within the triangle of support of the three feet in contact with the ground or on the diagonal of the two supporting feet. In the running walk the speed is faster (up to 25 km h^{-1}) and only one or two feet are on the ground at any one time. The running walk differs from other fast gaits (trot, pace and gallop) in that there is no period of suspension in which all four feet are off the ground at the same moment. It is the lack of a 'flight phase' and the consequent vertical movements of the centre of gravity that makes the running walk a very comfortable gait for the rider. For this reason it is generally assumed that it has been taught by man in those breeds in which it occurs¹².

Although the footfall sequence of the running walk is similar to that of the walk, it differs from it by the distance between hind and fore footprints and the duration of the periods of leg support. This is due to the greater extension of the legs in the running walk and the phase difference. In fast gaits the hind foot oversteps the fore foot to a variable extent, which can be expressed as a 'percentage of stride length'. Hence the running walk can be distinguished from the slow walk in a footprint trail (Fig. 2). The comparison of the fossil footprints of *Hipparion* with those of domestic recent horses revealed that *Hipparion* was moving in running walk (Figs 2, 3). The adult animal of trail *a* appeared to have travelled relatively fast (Icelandic horses producing an equivalent pattern of footprints travelled with a mean speed of 15 km h^{-1}); its hind foot overstepped the fore foot by 31% of the stride length. The adult of trail *b* appeared to have moved at a more moderate speed, estimated to average 10 km h^{-1} ; its hind foot overstepped the fore foot by 27% of the stride length. The young *Hipparion* of trail *c* reached with its hind foot 32% over its fore foot. It thus had a greater leg extension than the adult *Hipparion* of trail *b*, which together with the greater frequency of leg movement than the larger animal indicates that it was probably moving at the same speed as the adult. The spatial relationship between trails *b* and *c* is very similar to the criss-cross pattern of trails produced in living horses by a mare and its foal.

In trail *b* the point where the side-toe imprints are followed by vague broad imprints are of special interest. According to

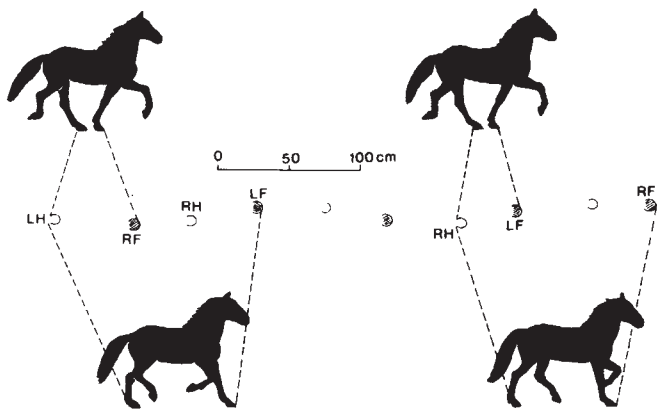


Fig. 3 The running walk: the four-beat alternation of diagonal and lateral leg support, in the sequence right front (RF), LH, LF and RH. The footprint pattern in the centre of the figure is trail *b* made by an adult *Hipparion*: its footfall sequence corresponds to that of a domestic horse travelling in running walk. The third horse from the left of the figure is in that stage of motion, corresponding to *Hipparion*, when it was sliding and being supported by the side toes of its right hind leg.

the footfall sequence the animal was sliding on its left fore foot, which left a broad and vague imprint (Fig. 3, third horse). The animal seems to have lost balance and compensated for this by the use of the opposite (diagonal) leg, (that is, the right hind leg) and shifting of the centre of gravity. This leg thus bore extra weight and was bent deeper through the fetlock of its main toe so that the side toes came in contact with the ground. The side toes, which were tightly attached to the main toe by ligamentous connections¹⁴, prevented overextension of the fetlock of the main toe. In *Hipparion* the 'springing mechanism' (that is, the assemblage of tendons in the foot in which elastic energy can be stored¹⁵) was apparently not as fully developed as in the main toe of modern horses¹⁶.

The fossil horse, however, had side toes, the elastic tendons of which could have actively contributed to the forward impulse. Hence, although the Laetoli soil was slippery due to the falling ash rain, *Hipparion* managed to balance itself and appears to have been able to pursue its route at constant speed. The importance of this analysis for understanding the evolution of equine gaits is twofold. The opinion that the running walk is an 'artificial and man-taught' gait is not supported by the results of this study. Young foals of several domestic breeds perform this gait in semi-wild conditions (E.R., unpublished observations), suggesting the ability to select this gait is a natural one in living equids.

Living horses are capable of selecting both lateral and diagonal gaits during locomotion, whereas most living mammals select only one or the other¹⁷. The more familiar diagonal gaits are the trot (a two-beat gait) and the transverse gallop (a three-four beat gait), but equids are also capable of selecting the lateral equivalent of these gaits, the pace and the rotary gallop (such gaits are typical of long-legged ungulates such as camels, giraffes and certain antelopes). The walk and the running walk contain a mixture of lateral and diagonal legs moving alternately, in a four-beat rhythm.

The results of the present study indicate that a diversity of gaits existed among the fossil forerunners of modern horses. It is uncertain why some horse breeds are able to use the full range of gaits.

I thank Dr Mary Leakey and Dr Paul Sondaar for entrusting to me the *Hipparion* trails for study, Professor D. M. Badoux, Dr C. M. Janis, Dr A. A. Macdonald and Dr Sondaar for their valuable comments on the manuscript, Drs Riemersma, Dr Schamhardt, Professor Hartman, Dr Wentink and Drs Fentener van Vlissingen for support in Utrecht, Professor Auffenberg, Professor Webb, Dr Colahan, Dr Hussain, Dr Piotrowski, Dr

Williamson, Dr Woods and Professor Perrin, horse breeders in the USA and The Netherlands, Dr A. C. Voeten in particular, for fieldwork, Mr Harry Otter for the technical assistance, Professor Hermans, Drs M. Fennis, Drs Cas Renders and Mr C. J. Stuyling de Lange LL.D. This work was supported by the Utrecht-Gainesville exchange program and the Mijnbouwfonds in Delft.

Received 14 July 1983; accepted 3 January 1984.

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Reciprocal food sharing in the vampire bat

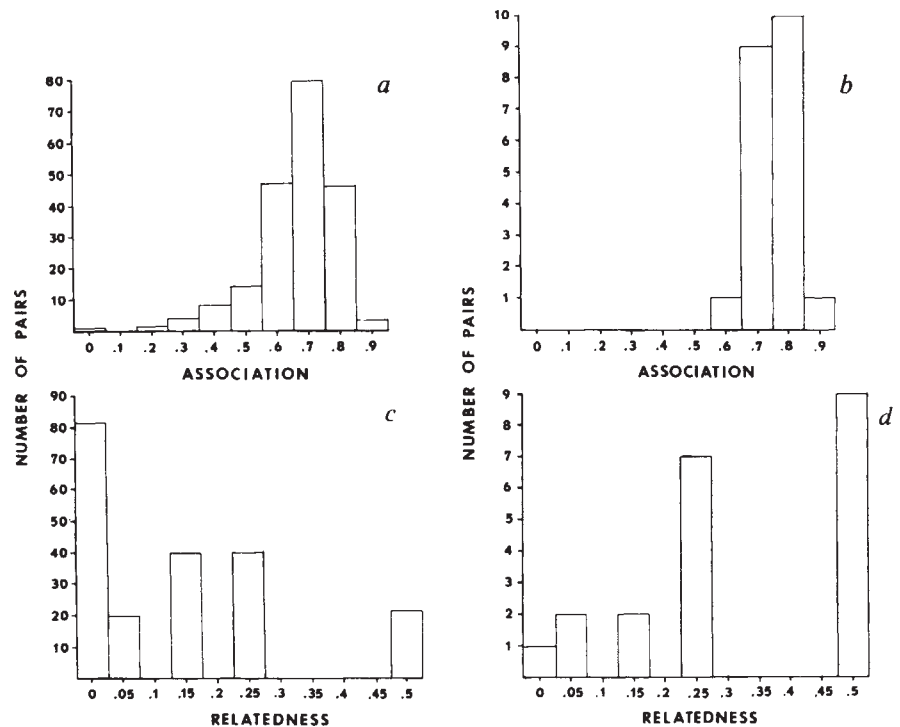
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Behavioural reciprocity can be evolutionarily stable¹⁻³. Initial increase in frequency depends, however, on reciprocal altruists interacting predominantly with other reciprocal altruists either by associating within kin groups or by having sufficient memory to recognize and not aid nonreciprocators. Theory thus suggests that reciprocity should evolve more easily among animals which live in kin groups. Data are available separating reciprocity from nepotism only for unrelated nonhuman animals⁴. Here, I show that food sharing by regurgitation of blood among wild vampire bats (*Desmodus rotundus*) depends equally and independently on degree of relatedness and an index of opportunity for reciprocation. That reciprocity operates within groups containing both kin and nonkin is supported further with data on the availability of blood-sharing occasions, estimates of the economics of sharing blood, and experiments which show that unrelated bats will reciprocally exchange blood in captivity.

The primary social unit of the vampire bat is the female group. During a 26-month (September 1978-February 1983) study in northwestern Costa Rica, 184 of the estimated 200 *D. rotundus* encountered inside 14 hollow tree day roosts at one site were netted and uniquely marked with wing bands. Infants were matched to mothers by direct observation of nursing or parturition. Weekly roost censuses revealed that adult females can be assigned to one of three groups, each consisting of 8-12 adults and their dependent offspring, according to which trees they visit. Members of each group moved between 2-5 day roosts; subsets of 2-4 females and young often roosted in different trees. Each of 17 males which attained 1 year of age left their natal groups while all 11 yearling female offspring were recruited into their maternal groups. Consequently, female groups contain some close relatives. Exceptions are due to the recruitment of, on average, one unrelated female per group every 2 yr. I estimated relatedness between females from path analysis⁵ of

Fig. 1 Data used in the logit analysis. The recipient in each of 21 regurgitations in which mothers did not feed their nursing young was matched to all potential donors in the roost to obtain the frequencies of association and relatedness for potential donor-recipient pairs graphed in histograms *a* and *c*, respectively. The indices computed between blood-sharing pairs, tallied in panels *b* and *d*, indicate that regurgitations occur predominantly among frequent roostmates and close relatives. The significance of these patterns was determined with a stepwise logistic regression in which relatedness entered first ($\chi^2_1 = 25.6$, $P < 0.001$) and the association entered second ($\chi^2_1 = 9.0$, $P < 0.003$). Neither the interaction of relatedness and association nor a sampling effect variable, regurgitation number, had sufficiently low probabilities ($P < 0.1$) to enter into the regression²⁰. Contingency table analyses showed that the frequency of regurgitation was independent of the age of either sex as well as the reproductive condition of females for both donors and recipients; however, females received blood more often than males ($\chi^2_1 = 5.58$, $P < 0.025$) even though donor sex was also independent of regurgitation frequency.



matrilateral pedigrees; observations and electrophoretic exclusion analyses indicated that paternity and inbreeding made negligible contributions to relatedness (manuscript in preparation). In the one group where pedigrees were extensive, the average coefficient of relatedness among adult females in 1981 was 0.11 (s.d. = 0.17, $n = 11$); estimates for relatedness (to be reported elsewhere) based both on electrophoretic data from other groups and simulations gave similar, but usually even lower, values.

During 400 daylight hours of focal animal sampling⁶ within roosts, 110 cases of regurgitation (mean duration = 63.0 s, s.d. = 57.6 s) were observed. Seventy-seven of these feedings occurred between a mother and her nursing offspring. In 21 of the other 33 donations, the degree of relatedness and a measure of association⁷, an index of opportunity for reciprocity, were known between the recipient and every other bat in the tree. (The association index is $2N_i/(N_1 + N_2)$ where N_i represents the number of times both bats were observed together until the day of regurgitation and N_i is the total number of times bat *i* was observed.) By logit analysis⁸ of these data (Fig. 1) both related-

ness and association significantly predict regurgitation independent of their correlation. The relative effects of the variables, as measured by the standardized regression coefficients⁹ (-1.25 , s.e. = 0.48, for association and -0.96 , s.e. = 0.33, for relatedness), are approximately equal. These results indicate that regurgitations between distant relatives only occur between animals that have been frequent roostmates, as expected where reciprocity occurs. For reciprocity to persist among *D. rotundus*, however, three conditions¹ need to be fulfilled: (1) enough repeated pairwise interactions must occur to permit role exchanges and ensure that a net benefit accrues to all donors; (2) the benefit of receiving aid must exceed, on average, the cost of donating; and (3) donors must be able to recognize and not feed previous recipients that fail to reciprocate.

Several aspects of female *D. rotundus* natural history act together to create repeated opportunities for exchanging blood. Bats that fail to obtain a blood meal are usually fed by roostmates. Five of 8 bats captured before feeding and released into their roosts at dawn were subsequently given blood by a group member, whereas six bats captured at the same time but after

Table 1 Blood sharing among captive *Desmodus rotundus*

Starved bat	Donor bat(s)	Potential reciprocators*	Reciprocal feeding?	Probability of exchange†	
				Cage	Population
ROG(S)	OYR(S)	—	—	—	—
OO(L)	GB(L)	—	—	—	—
WB(L)	OO(L)	—	—	—	—
GB(L)	WB(L)	OO	No	4/5	2/3
OYR(S)	ROG(S)	ROG	Yes	1/7	1/3
YBR(S)	—	—	—	—	—
GRB(L)	WB(L)	—	—	—	—
ROG(S)	GRB(L)	OYR	No	5/6	—
OO(L)	WB,GB(L)	WB	Yes	1/5	1/3
WB(L)	GRB,GB(L)	GRB,GB	Yes	1/15	1/3
YO(S)	OYR(S)	—	—	—	—
GRB(L)	WB(L)	WB,ROG	Yes	2/7	1/3

Starved and donor bats are ordered according to the chronological sequence of blood sharing; population of origin is indicated in parentheses.

* Bats which have been fed by the recipient but have not yet returned any blood.

† The probability that the observed donation would occur with a potential reciprocator by chance given the number of potential donors either in the cage or from the same population. The combined binomial probability of witnessing as many or more such reciprocal exchanges, if any adult in the cage is considered a potential donor, is 0.009; if donors must come from the same population the probability is 0.045. Bat YBR was an adult male; ROG was near parturition; YO had a nursing infant; and GB was the grandmother of OO.

feeding received no blood when released ($P=0.003$, binomial probability). Thus, reciprocal blood sharing can occur only if failure to obtain a blood meal is not synchronized in the population. The following shows that only age influences feeding success. At the main study site, La Pacifica, 23 of 121 bats, captured on 22 nights returning to roosts too late for additional foraging, weighed less than pre-fed weights determined on previous captures and were assumed therefore not to have fed. Using the same criteria at another study site, Santa Rosa, I found that 86 of 477 bats also failed to feed on 31 nights. Contingency table analyses using three-way G tests showed no effect of sex, population, reproductive condition or Moon phase on feeding success. But when population and sex categories are pooled, 33% of 258 bats less than 2 yr of age failed to feed while only 7% of the 340 older bats did not obtain blood meals ($G=45.3$, $P<0.005$). These data are not biased by a few repeatedly unsuccessful foragers; at both sites the frequency of unsuccessful feeding trips for individuals captured two, three or more times did not differ significantly from expected frequencies after controlling for age effects. When considered with the longevity of females and stable composition of groups, these data indicate that numerous opportunities both to share and to receive blood exist. Females as young as 8 months of age were observed to donate blood (personal observation) and can live for 18 yr¹⁰. Annual mortality is sufficiently low among adults (24%) for females to spend long periods together. For example, seven of the nine adult females present in one group in 1980 were still roosting together in 1982; in another group one pair of females marked by T. Fleming (personal communication) in 1970 roosted in the same area in 1981.

Vampire bats fulfill the second condition for reciprocity since their weight decays exponentially following a meal (Fig. 2a and ref. 11). To predict the time remaining to a fasting bat before death, weight loss attributable to post-feeding diuresis¹² can be subtracted from overall weight loss to produce a curve (Fig. 2b) which is due to metabolic and evaporative water losses. The concave downwards shape of this curve enables a donor to transfer weight by regurgitation and lose less time before reaching starvation than a recipient gains (Fig. 2b). The amount of blood transferred need not translate linearly into units of survivorship; it is sufficient that a blood donation increase the time until starvation sufficiently for a bat in need to forage at least once more. Observed blood transfers support this proposition. I estimated from a calibrated weights loss curve that one captive bat which received blood for 400 s gained about 12 h, assuming that fluid is transferred at drinking rates¹³. For comparison in the wild, one donation lasted 390 s.

The third condition for reciprocity requires that nonreciprocating bats can be identified and refused aid. I conducted experiments on captive animals which confirmed part of this requirement; unrelated but closely associated bats are capable of sufficient recognition to reciprocally exchange blood. The study group was created by housing four La Pacifica adult females from one roost tree with three adult females, one infant and one adult male from a cave 50 km away at Santa Rosa, in a cage 60 × 30 × 30 cm. No two bats shared a common ancestor for at least three generations except a grandmother-grand-offspring pair from La Pacifica. Association of females within populations was high but that between the male and females was low. To create a need for regurgitation, one bat was removed each evening before feeding and the remaining bats were given access to blood for 2 h. Two to twelve hours later the hungry bat was reintroduced and all ensuing interactions between individuals during the next 2 h were observed through an image intensifier with IR illumination and recorded. Each evening a different bat was chosen at random without replacement until every adult had been starved. All bats were starved at least twice during the experiment and both body and blood meal weights were recorded daily. Prior to the experiment weight loss curves such as shown in Fig. 2 were obtained for each adult bat and fitted with negative exponential functions to predict survival time.

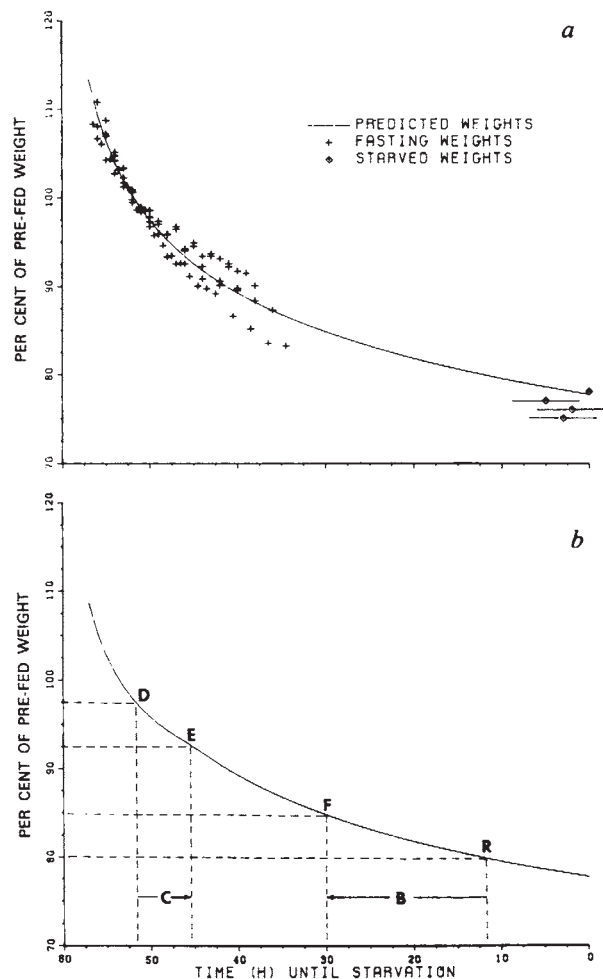


Fig. 2 Post-feeding (≥ 2 h) weight losses of one adult male and five adult female captive *D. rotundus* expressed as per cent of weight when captured at 1900 h (a). This weight was used as a referent because of its convenience and constancy; mean coefficient of variation for adult males captured three or more times was 2.85% (s.d. = 1.17, $n=11$). To control for differences in meal sizes, the times elapsed since feeding that corresponded to each bat's 100% pre-fed weight (other values produced similar curves) were matched and a negative exponential function¹¹ ($W=130.25t^{-0.126}$, $r^2=93.0$, $n=82$, $P<0.001$) was fitted by least-squares estimation. The weights of four bats that starved accidentally in a prior experiment were not entered in the regression but are included, with error bars to indicate uncertainty as to the exact time of death, in a to show the predictive ability of the function in the extrapolated region and that death does occur near 75% of pre-fed weight. Weight loss attributable to diuresis¹² 15 h post-feeding was subtracted from the fitted curve in a to obtain the predictive curve in b. Because fluid is absorbed in the stomach approximately at the rate that urine is excreted¹³, blood meal consistency changes little 2 h after feeding. Therefore, a donation of 5% of pre-fed weight when at weight D should cause a donor to lose C h but provide B h to a recipient at weight R. The recipient benefit always exceeds the donor's cost when $E>F$.

Blood was donated preferentially to individuals in dire need from the same population. For each of the 13 observed regurgitations the recipient would have reached minimum viable weight in less than 24 h (mean 13.2 h, s.d. = 4.7 h), if it had not received blood. On the other hand, donors could have expected to survive at least 24 h (mean = 39.9 h, s.d. = 15.5 h). Consequently, trials in which the starved bat had received no blood and had more than 24 h left before starvation were excluded from the following analyses. Twelve of the 13 ($P=0.002$, binomial probability) regurgitations (mean duration = 25 s, s.d. = 25 s) occurred between bats from the same population (Table 1). To test whether or not these feedings occurred independently of prior

blood sharing, each trial in which a donor fed a starved bat was compared with the subsequent trial in which the donor was starved. Starved bats which received blood later reciprocated the donation significantly more often than expected had exchanges occurred randomly (Table 1). These results do not depend on donors having more blood to share than nondonors. The rank of the donor with regard to its expected number of hours left before starvation was not significantly different from the median rank of bats within the cage or population ($P > 0.15$, Kolmogorov-Smirnov test). As expected, the only bat with low association, YBR male, was not fed even when in need. These results are very difficult to reconcile with any interpretation of food sharing based solely on kin selection.

Two recent reviews^{14,15} overemphasize suspected instances of reciprocity which occur among unrelated animals. The results described here indicate that reciprocity can occur within groups that contain relatives. Further study of other food-sharing mammals¹⁶⁻¹⁹ as well as other altruistically behaving animals which live in kin groups may reveal that reciprocity is more important in maintaining altruism than has previously been acknowledged.

This project was funded by NSF grant DEB-8001165 to J. Bradbury and NIH training grant GM-0724008 to the Department of Biology, UCSD. I thank D. Bolger, M. Jones, T. Lamp and R. Weiss for field assistance and J. Bradbury, R. Gibson and M. Taper for suggestions and comments.

Received 19 July; accepted 13 December 1983.

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Bipolar cells in monkey retina selective for the cones likely to be blue-sensitive

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Bipolar cells are a class of retinal interneurons with dendrites in the outer plexiform layer that contact photoreceptors, and axons terminating in the inner plexiform layer¹ where they convey the centre responses of ganglion cells^{2,3}. In the monkey, many of whose ganglion cells respond to stimuli selective⁴ for each of the three different cones^{5,6}. There are five types of cone bipolar cells: flat and invaginating midget⁷⁻⁹; diffuse, flat⁷ and invaginating cone^{7,10}; and giant, bistratified¹¹. Although many of the monkey's ganglion cells are specific for one of the three different cone mechanisms, none of the bipolar cells are known to connect to the morphologically identified counterparts of the different spectral types of cones as in teleost retina¹²⁻¹⁵. Here,

however, I describe a bipolar cell found in Golgi preparations of the rhesus monkey retina which displays an apparently selective and patterned distribution of its dendritic contacts with cone pedicles in the outer plexiform layer. The intercone spacing of dendritic contacts and their distribution match the intercone spacing and proportion of cones thought to be blue-sensitive^{16,17}.

Thirty-six retinas of 19 adult, male and female, rhesus monkeys (*Macaca mulatta*) were Golgi impregnated as described elsewhere¹⁸. Most neurones were observed in whole mounts in which there was no linear shrinkage. Selected cells were radially sectioned, and the preparations were studied by light microscopy. A bipolar cell type which appeared similar to invaginating midget bipolar cells was found. It possessed a small dendritic cluster, smaller than the diameter of a cone pedicle, and an axon that terminated low in the inner plexiform layer (IPL) (Fig. 1a). However, it displayed many distinct features. Several primary dendrites arose from the apical surface of the cell body and coursed horizontally through the outer plexiform layer (OPL) (Figs 1a, 2). Some of these converged at a distance from the axis of the cell body and axon, and at the level of cone pedicles formed a small cluster of coarse dendritic terminals (Figs 1a, 2) identical in span to that formed by the invaginating midget bipolar cells (3.5 μm in the most central regions and up to 7 μm in peripheral retina). At 5 mm eccentricity these clusters were composed of nine dendritic terminals ($n = 10 \pm 1.41$ s.d.) The individual terminal dendrites, although too small to be measured accurately with a conventional ocular reticle ($< 0.5 \mu\text{m}$), appeared to be larger than those of the invaginating midgets. Some other dendrites of this new cell type ended wholly within the inner stratum of the OPL and did not contact any photoreceptor terminal (Figs 1a, 2). In the IPL, the axon descended to the innermost stratum adjacent to the ganglion cell layer, where it terminated as an elongate structure, with few branches, and with a total span ranging from central to peripheral retina of about 20-50 μm (Fig. 1a). Although this terminal arborization was broad in span, it was narrowly stratified in the innermost stratum of the IPL, and while ending in close proximity to the ganglion cell bodies, these axon terminals did not appear to touch or insert themselves between these cell bodies as did the axon terminals of rod bipolars⁷. Invaginating midget bipolar cells, however, had a single main dendrite which terminated in a 'bouquet'⁷ (Figs 1b, 2). At the same eccentricity (5 mm) where the newly identified bipolar cells had an average of nine dendritic terminals the 'bouquets' of invaginating midget bipolar cells had 19.8 dendritic terminals ($n = 10 \pm 3.31$ s.d.). Additionally the axon terminal of the invaginating midget bipolar cell, which ended in the inner half of the IPL, was broadly stratified but did not reach the innermost stratum and was narrow in span in the plane of the retina (Figs 1b, 2).

Serial resectioning of three of the newly identified Golgi-impregnated bipolar cells for electron microscopy demonstrated that those dendritic terminals which reached the level of the cone pedicles, invaginated these structures, and terminated as central elements at cone triads (Fig. 1c). Enough sections were recovered from two cells to find that the dendritic terminals occupied the central elements of 8 of 11 and 11 of 15 ribbon synapses respectively. Several of the 'blind' dendrites, those which did not reach the level of cone pedicles in the OPL, were followed to their termination in serial sections. There was no evidence that these processes continued, unimpregnated, to the photoreceptors.

These newly identified bipolar cells sometimes contacted two cones (Fig. 2), as did double-midget bipolar cells, but the two cones contacted by these new bipolar cells were always separated widely by approximately three intercone distances and were never seen to contact two adjacent cones as double-midgets did (Fig. 2). The smaller dendritic cluster of the pair which contacted two cones was often seen to overlap with the large cluster of one which contacted only one cone, and the dendritic clusters of these newly identified bipolar cells were not seen to overlap

Fig. 1 *a*, Radial section of a Golgi-impregnated, blue-cone bipolar cell. Several dendrites emerge from the cell body, course through the OPL and converge at their termination. One dendrite (arrow) ended freely in the OPL. $\times 610$. *b*, Golgi-impregnated invaginating midget bipolar cell found directly below the cell in *a* in the adjacent section. $\times 610$. Note particularly the differences in dendritic and axonal arborizations between *a* and *b*. *c*, Electron micrograph of a tangential section of the dendritic terminations of a blue-cone bipolar cell as central elements opposite several synaptic ribbons, SR, of a cone pedicle. $\times 12,600$.

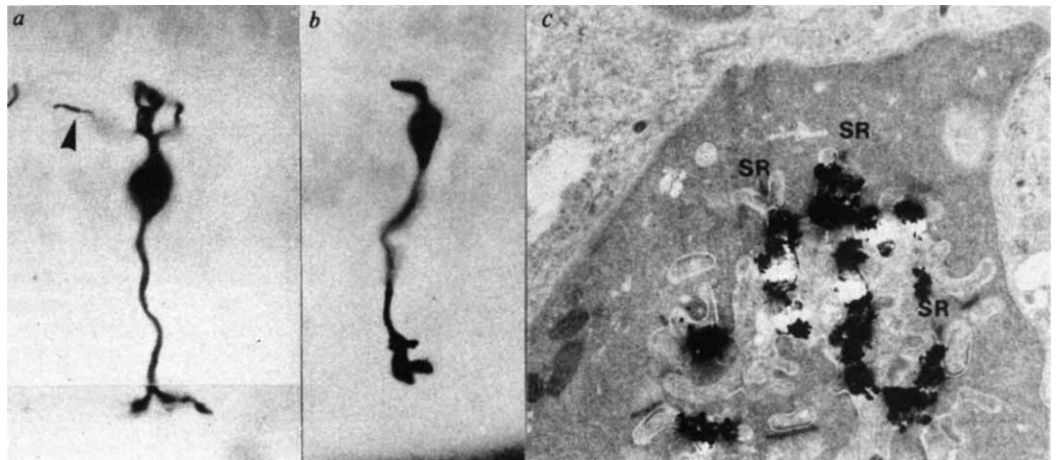


Table 1 Intercone spacing of blue-cone bipolar cell dendritic clusters

Distance from centre of fovea mm	Deg of arc*	Intercone distance of all cones (ICD) $\mu\text{m}(n) \pm \text{s.d.}$	Spacing of blue-cone bipolar cell dendritic clusters (BCD) $\mu\text{m}(n) \pm \text{s.d.}$	$\frac{\text{ICD}^2}{\text{BCD}^2}$ IN %
10	40	$17.1(79) \pm 3.88$	$67.3(3) \pm 1.53$	6.4
7	28	$14(30) \pm 1.86$	$53(3) \pm 6.24$	7
5	20	$14.4(26) \pm 1.96$	$48.2(5) \pm 10.8$	8.9
3	12	$10.65(55) \pm 1.83$	$34.6(3) \pm 2.51$	9.5
1.5	6	$7.65(52) \pm 1.08$	$24(4) \pm 4.08$	10.2
0.75	3	$5.46(46) \pm 1.0$	$27(2) \pm 4.24$	4.1

Intercone spacing and ratio of cone population contacted by blue-cone bipolar cells to the total cone population. Compare with data on staining of the likely blue-sensitive cones^{16,17}.

* Conversion of linear distance to visual angle according to Rolls and Cowey²⁶ where $1 \text{ mm} = 4^\circ$.

with those of midget bipolar cells. This suggests that they contact a uniform photoreceptor population different from that contacted by the midget bipolars. The several morphological distinctions between invaginating midgets and the newly identified bipolars include a notable difference in the spacing of the intercone contacts. Those that contact two cones represent one-third of the population of cells observed, and only a few cells had three dendritic clusters. The probable blue-sensitive cones in the macaque retina represented numerically the smallest population^{16,17}, that is, generally less than 15%, and this suggested that these newly identified bipolars contacted the blue cones. Evidence was obtained from the intercone spacing (Table 1). The measurements from 10, 7, 5, 3 and 1.5 mm of eccentricity all agreed well with both the intercone spacing and percentage of the presumed blue-sensitive receptors in baboon¹⁶ and with Procion yellow-stained cones in macaques¹⁷. The measurement and derived numbers from the fovea (0.75 mm eccentricity) agreed with the above-cited studies for the following reason. Photoreceptors in the primate fovea have long, obliquely oriented axons of up to 600 μm or more⁷ which displace their synaptic terminal in a peripheral direction from the inner and outer segments of the cone from which they originate. Thus these bipolar cells were contacting cones more centrally located than their synaptic pedicles most probably in the most central part of the retina where presumed blue-sensitive cones are rare or absent^{16,17}. Thus, from the intercone spacing of the dendritic contacts the photoreceptors were likely to be the blue-sensitive cones and the bipolar cells were probably selective for the blue-sensitive cones and could be termed blue-cone bipolar cells. Although, since any two cones of the same spectral sensitiv-

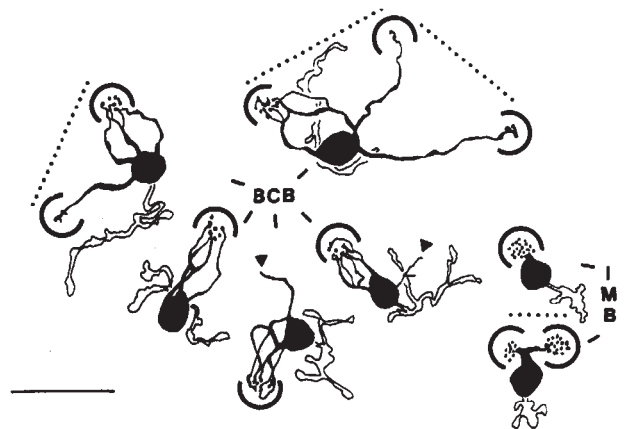


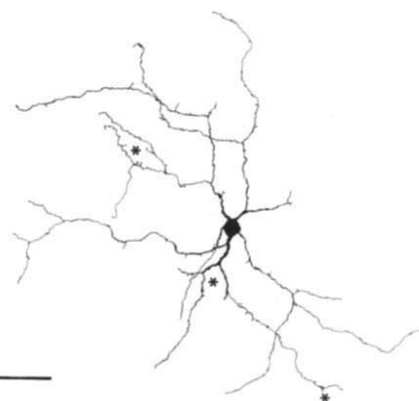
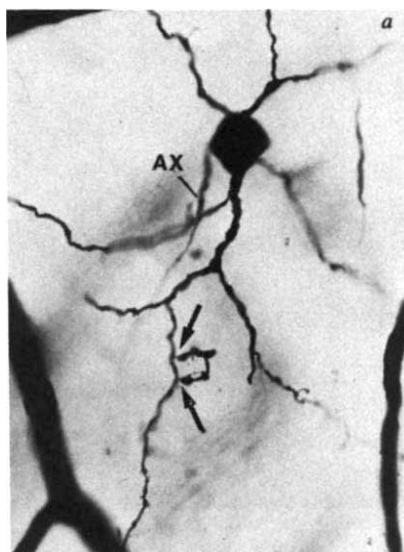
Fig. 2 Camera lucida drawing of the cell bodies and dendrites of several blue-cone bipolar cells (BCB) and two invaginating midget bipolar cells (IMB) in a whole, flat, Golgi-impregnated rhesus monkey retina 5 mm from the centre of the fovea. $\circ$, Clusters of dendritic terminals that contacted cones, dotted lines extend between clusters of single cells which contacted more than one cone. Arrowheads point to 'blind' dendrites of BCB which terminated in the OPL below the cone pedicles. The axon arborizations are outlined. Scale bar, 25 μm .

ity could be found the same distance apart as the dendritic contacts of the blue-cone bipolars, it is possible that these cells are not selective for blue-sensitive cones, this seems most unlikely, as the red- and/or green-sensitive cones can be found next to one another¹⁶ and, therefore, some of the blue-cone bipolar cells contacting two (or more) cones would have dendritic clusters spaced by one intercone distance. Of all the bipolar cells of this type, I have found in the 36 retinas, none had clusters spaced by one intercone distance.

The finding that the blue-cone bipolar cells have fewer dendritic terminals than invaginating midget bipolar cells and that the cones they connected with have fewer triads than those which connected with invaginating midgets suggests that the blue-sensitive cone synaptic pedicles could be identified by conventional electron microscopy since they have fewer synaptic ribbons. The unstained central elements in the blue-sensitive cone pedicles found here could be occupied by one of the small overlapping dendritic clusters of the blue-cone bipolars which contacts two cones, or, as proposed for unimpregnated central elements at cones connected to invaginating midget bipolar cells by the dendrites of the diffuse invaginating cone bipolar cell¹⁰.

Midget bipolar cells are of two types that end either at ribbon synapses or form wide-cleft basal junctions, but the blue-cone bipolar cells were only of the type ending at ribbons; no bipolars which formed wide-cleft basal junctions with cones or had axons

Fig. 3 *a*, Light micrograph of a ganglion cell with a dendritic arborization in the innermost stratum of the IPL as viewed in a whole, flat preparation. Spines projected from one of the dendrites (arrows) to the axonal ending of a blue-cone bipolar cell suggesting a connection between the blue-cone bipolar cells and this type of ganglion cell. AX, axon. $\times 448$. *b*, Camera lucida drawing of the cell photographed in *a*. Asterisks indicate positions where three Golgi-impregnated, blue-cone bipolar cell axon terminals including that of *a* (large asterisk) contacted dendritic spines of this ganglion cell. Scale bar, 75 μ m.



branching in the outer strata of the IPL were found to have a similar dendritic pattern to the blue-cone bipolar cells. As the presumed blue-cone bipolar cells were postsynaptic at ribbon junctions, and since ribbon and/or narrow-cleft junctions are thought to mediate 'on' responses in bipolars^{15,19} and transmission from bipolars ganglion cells is most probably excitatory^{20,21}, blue-sensitive ganglion cells should be 'on-centre'. In monkey retina nearly all blue-cone ganglion cell responses recorded are 'on-centre'^{22,23}. The presumed blue-cone bipolar cells probably mediate the blue, on-centre pathway to retinal ganglion cells in the IPL of rhesus retina. As the axons of the blue-cone bipolar cells ended below the dendrites of the inner midget ganglion cells, and as their span was wider than that of a midget ganglion cell's dendritic arborization, midget ganglion cells are not likely to carry blue-cone responses. However, a type of retinal ganglion cell, not previously described in Golgi-impregnation studies of the primate retina^{7,8} and clearly not a midget ganglion cell, arborized in the innermost stratum of the IPL and had dendritic spines projecting to axon terminals of the blue-cone bipolar cells (Fig. 3); this could form part of the blue circuitry of the retina.

Many unusual electrophysiological and psychophysical^{24,25} features distinguish the blue-sensitive system from the red- and green-sensitive cone pathways, and the identification of a specific part of the blue-sensitive retinal circuitry should promote understanding of the neuronal bases for these differences.

Spectral sensitivity of a novel photoreceptive system mediating entrainment of mammalian circadian rhythms

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Environmental light cycles are the dominant synchronizers of circadian rhythms in the field, and artificial light cycles and pulses are the major tools used in the laboratory to analyse properties of circadian systems¹⁻⁴. It is therefore surprising that few studies have analysed the physical parameters of light stimuli that affect circadian rhythms. There have previously been no spectral sensitivity measurements for phase shifting the circadian rhythms of mammals and only two preliminary reports on the wavelength dependence of this response exist^{3,4}. Using the magnitude of phase shift caused by a single 15-min pulse of monochromatic light given 6 h after activity onset, we have now characterized the spectral sensitivity of the photoreceptors responsible for phase shifting the locomotor rhythm of the hamster (*Mesocricetus auratus*). The sensitivity curve for this response has a maximum near 500 nm and is similar to the absorption spectrum for rhodopsin. Although the spectral sensitivity is consistent with a rhodopsin-based photopigment, two features of the photoreceptive system that mediates entrainment are unusual: the threshold of the response is high, especially for a predominantly rod retina like that of the hamster, and the reciprocal relationship between intensity and duration holds for extremely long durations (up to 45 min). These results suggest that the photoreceptive system mediating entrainment is markedly different from that involved in visual image formation.

While all other vertebrates have extraretinal photoreceptive input to the circadian system⁵, in mammals the photoreceptors for entrainment and phase shifting are located in the eye^{6,7}. A

Received 15 July; accepted 17 December 1983.

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specialized retinal projection terminating in the suprachiasmatic nuclei of the hypothalamus conveys photic information for these processes^{8,9}. Because the location of photoreceptors and their output pathways are known in mammals, they are the vertebrates of choice for study of the effects on the clock of varying the intensity and spectral qualities of light. The responses to light cycles and pulses of the circadian system of the golden hamster have been extensively studied^{10,11}. Its locomotor rhythm is easily recorded and its phase shifting response is precise and repeatable.

The photoreceptive assay used the magnitude of phase shift caused by a single 15-min pulse of monochromatic light. Male golden hamsters (Lakeview LVG outbred) were individually housed in running-wheel cages for locomotor activity recording. After a 7-day acclimation period in a light/dark cycle, animals were maintained in constant darkness for 7 days, then exposed to a 15-min pulse of monochromatic light and finally returned to constant darkness for 14 days (Fig. 1a). Monochromatic light pulses were administered at circadian time 18, the phase of the rhythm at which maximum phase advances occur (Fig. 1b). The uniformity of the response at this phase is shown in Fig. 1c. In these conditions, the maximal phase shifts obtained with white light averaged 2.4 h for 1-h pulses and 2.5 h for 6-h pulses.

To characterize the spectral properties of the photoreceptors that mediate entrainment, the effect of varying the intensity of monochromatic light upon the magnitude of phase shift was determined at three different wavelengths. Light pulses at 476 nm and 515 nm were more effective in causing phase shifts than were pulses at 574 nm (Fig. 2a). At each wavelength the amount of phase shift increased with intensity. The response-intensity relationship, which was pseudolinear at low intensities and appeared to saturate at high intensities, could be described by a modification of the rectangular hyperbola or Michaelis-Menten equation (see Fig. 2a). Although the sensitivity of the response varied with wavelength, the response-intensity relationship was similar at the three wavelengths (Fig. 2a) and therefore was consistent with the principle of univariance¹².

To determine the spectral sensitivity of the phase-shifting response, the quantum intensity required to produce a half-maximal response was determined at seven wavelengths, and the reciprocals of these values (normalized) were plotted to yield the action spectrum (Fig. 2b). The spectral sensitivity curve has a maximum near 500 nm; it drops off gradually at shorter wavelengths and more sharply at longer wavelengths. The hamster retina contains a single, extractable visual pigment with a maximum at 502 nm¹³. There is reasonable agreement between the sensitivity curve and the Dartnall nomogram for the visual pigment in hamster retina (Fig. 2b). At short wavelengths the curves are indistinguishable; however, at long wavelengths the spectral sensitivity curve falls off more sharply than expected. The disparity at long wavelengths could be due to a screening pigment or to the contribution of another visual pigment. Because long-duration, intense light pulses were required to produce phase shifts, some light adaptation may have occurred and could have been responsible for modifying the sensitivity at long wavelengths. Granit¹⁴ reported that the spectral sensitivity curves of single units in the rat retina were consistent with rhodopsin after dark adaptation, but were narrower after light adaptation.

Two features of the photoreceptive system that mediates entrainment are quite unusual. First, the threshold for producing a phase shift is very high. At 515 nm the intensity required to produce a half-maximal response is about 6 log units above the threshold for human vision¹⁵. Second, the reciprocal relationship between intensity and duration in producing an equal response holds for extremely long durations. In rods, reciprocity holds for durations up to 1–3 s¹⁶, and in cones for durations up to 100 ms¹⁷. In marked contrast, reciprocity holds for at least 45 min in the phase-shifting response of the hamster. In experiments in which the total number of quanta delivered in a pulse was held constant and the duration was varied from 4 to 45 min, the magnitude of the phase shift was constant (unpublished

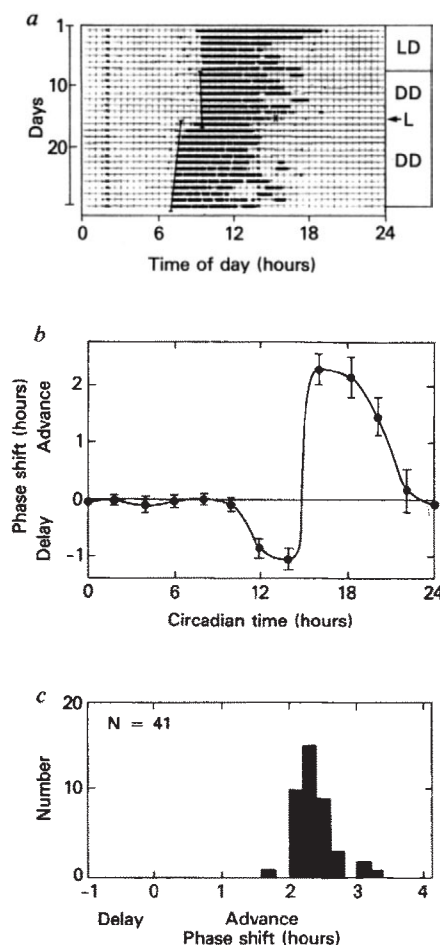


Fig. 1 Effect of short light pulses on the phase of the circadian locomotor rhythm in hamsters. **a**, Locomotor activity record illustrating the phase-shifting effect of a single 15-min monochromatic light pulse. Each horizontal line represents the activity record from a 24-h day, and successive days are plotted below one another. Light schedule: on days 1–7 a 14-h light/10-h dark cycle (LD) with lights off at 0900 h and on at 1900 h PST; on days 8–15 complete darkness (DD); on day 15, a 15-min 511-nm monochromatic light pulse (L) at the time indicated by the asterisk; and on days 15–29 complete darkness. Steady-state phases of the onsets of activity before (days 8–16) and after (days 16–29) the light pulse are indicated by the two lines. The phase shift was determined by extrapolating both phase estimates to day 16 and calculating their phase difference. **b**, Phase response curve: The dependence of the magnitude and direction of the phase shift on the phase of the rhythm at which the stimulus was applied. Onset of activity, defined as circadian time 12 by convention, was used as a phase reference. Each point represents the mean $\pm$ standard deviation of six animals plotted in 2-h bins indicating the onset of a 60-min white fluorescent light pulse (350 lx). Data were obtained by J. S. T., F. C. Davis and M. M. **c**, Frequency histogram of the magnitude of phase shifts caused by a 60-min white fluorescent light pulse given at circadian time 18. The mean phase shift for the group was 2.35 ± 0.32 h (mean $\pm$ s.d.).

Methods: Monochromatic light exposure: hamsters were transferred in complete darkness to a cylindrical chamber (10.0 cm diameter $\times$ 5.7 cm high). Monochromatic light was projected onto a 12-cm diameter opal glass disk which formed the roof of the chamber. Light from a 500-W tungsten lamp (G. E. CZX/DAB) was collimated by a series of condenser and projection lenses. A manual shutter was used to control pulse duration. Light was filtered with narrow band interference filters (Balzers and PTR Optics, 9–16 nm half-bandwidth) and intensity was adjusted with neutral density filters (Bausch and Lomb). Light intensities were measured directly with a Gamma 2400 quantum photometer using a Gamma RS70-2 standard lamp for calibration. Total radiance in photons $\text{cm}^{-2} \text{s}^{-1} \text{sr}^{-1}$ was calculated by integrating the spectral radiance of each filter in 5-nm steps.

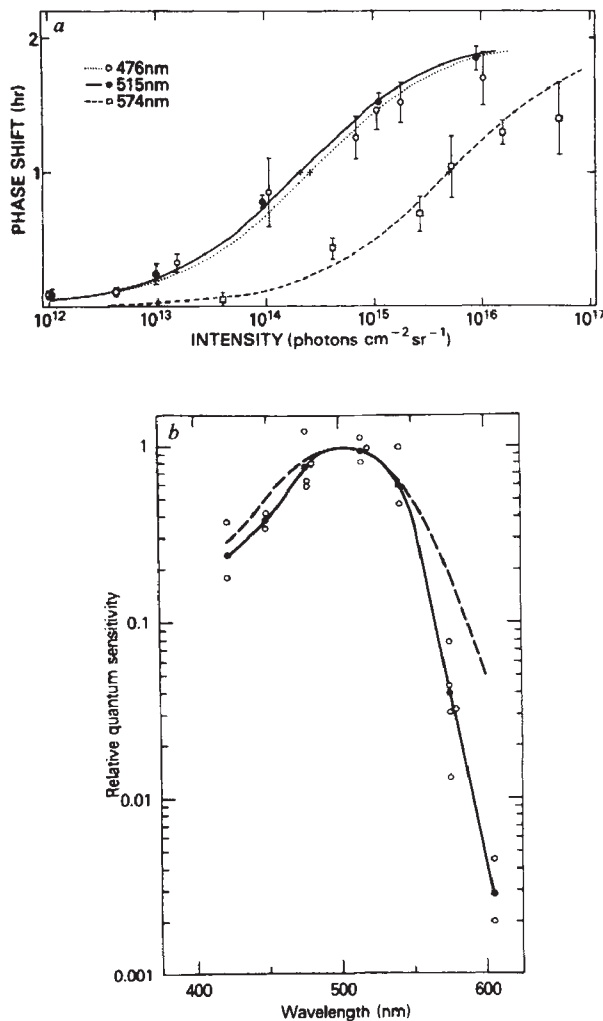


Fig. 2 *a*, Magnitude of phase shift in response to 15-min monochromatic light pulses of increasing intensity for three different wavelengths (476, 515, 574 nm). The data from the most effective wavelength, 515 nm, are fitted by the equation $r/r_{\max} = i^n/(i^n + i_0^n)$, where r represents magnitude of phase shift (h), with maximum $r_{\max}$; i represents intensity (photons $\text{cm}^{-2} \text{sr}^{-1}$) with half-saturating intensity i_0 ; and n is the slope of the relation. For the 515-nm curve (solid line) $r_{\max} = 2.0$, $i_0 = 2.1 \times 10^{14}$ and $n = 0.7$. The same curve displaced to the right fits the data from 476 nm (dotted line with $i_0 = 2.6 \times 10^{14}$) and 574 nm (dashed line with $i_0 = 5 \times 10^{15}$). The data obtained at 476 nm and 574 nm do not deviate significantly from the equation fitted to the 515-nm data. *b*, Spectral sensitivity for phase shifting the hamster circadian system. Relative quantum sensitivity was determined as described by Naka and Rushton¹² and Baylor and Hodgkin²². The data were normalized to a value of unity at 500 nm, which corresponds to 2×10^{14} photons $\text{cm}^{-2} \text{sr}^{-1}$. Eye-fitted curve through closed circles denotes estimates (based on 10–40 animals at each wavelength) determined from curve fitting as described in *a*. Open circles represent estimates from the mean phase shift of groups of three to six animals at a single intensity that produced responses in the pseudolinear portion of the intensity–response curve (that is, phase shifts at 20–80% of maximum response). Broken curve is a Darnall nomogram for a retinal₁-based pigment with a maximum at 502 nm²³.

results). This result implies that the clock's photoreceptive system is capable of integrating, or counting, photons over extremely long durations without becoming nonlinear in its response. The high threshold and the linear properties of the hamster photoreceptive system suggest that even when clock photoreception is performed by the image-forming eyes, the rules that govern it and perhaps even the retinal cells that mediate it may be very different from those involved in image formation.

Because the hamster retina is predominantly composed of rod photoreceptors and because the spectral sensitivity of the phase-shifting response is similar to that of rhodopsin, our results are consistent with the hypothesis that rod photoreceptors contribute to this response. If this is correct, however, a mechanism must exist for reducing the sensitivity of the circadian system to photic input because the threshold we have found for phase shifting is much higher than the threshold for scotopic (rod)

vision. In principle, the attenuation of photic input could occur at any point between the photoreceptor and the circadian pacemaker. Alternatively, the high threshold could be achieved at the receptor level by using cones. As reported in other rodents¹⁸, cone-like photoreceptors exist in the hamster retina in small numbers (N. Buyukmihci and A. H. Bunt, personal communications) and the possibility remains that a cone photoreceptor with a peak sensitivity near 500 nm could mediate the response.

In rodents, the suprachiasmatic nuclei (SCN) of the hypothalamus are necessary for the generation of circadian rhythmicity^{8,9}. These nuclei receive direct retinal input via the retinohypothalamic tract^{8,9}. Retrograde labelling of ganglion cells projecting to the SCN shows that the cell bodies are distributed diffusely over the hamster retina¹⁹, and electrophysiological recordings from SCN neurones reveal a high photic threshold and large diffuse receptive fields²⁰. The anatomical and physiological data strongly suggest that a non-visual photoreceptive system with novel properties subserves the entrainment process in mammals. That such non-visual photoreceptive systems may be of hitherto unsuspected importance for human beings is suggested by the high intensities of light required to suppress melatonin synthesis by the human pineal gland²¹.

We thank Dr D. J. Hudson and B. A. Liepe for their assistance. This work was supported by NIH grants HD 13162 (to M.M.) and 5T32 GM 07257 (to J.S.T.), and Am. Ass. Univ. Women fellowship to P.J.D. This paper is dedicated to Professor Colin S. Pittendrigh on the occasion of his 65th birthday.

Received 13 September; accepted 12 December 1983.

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Two types of mutants affecting voltage-sensitive sodium channels in *Drosophila melanogaster*

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Voltage-sensitive sodium channels have a key role in the genesis of propagated action potentials¹⁻⁴. Mutations that affect these channels might be used like specific pharmacological agents in studies of channel structure and regulation. We have found that mutations which cause a reversible, temperature-induced paralysis in the fruit fly *Drosophila melanogaster* often affect the voltage-sensitive sodium channel. Using ³H-saxitoxin binding to membrane preparations, we have now identified two types of presumptive sodium channel mutants. One mutation, seizure^{ts-2} (*sei*^{ts-2}), appears to alter saxitoxin-binding sites structurally. The second, no-action-potential (*nap*^{ts}) (refs 5, 6), reduces the number of saxitoxin-binding sites, but appears not to alter the receptor structure.

Electrophysiological analyses⁵⁻⁷ have shown that several temperature-sensitive paralytic mutants of *Drosophila* display a temperature-induced loss of action potentials, and could therefore be mutants with sodium channel alterations. We have examined crude neural membrane preparations from 11 different temperature-sensitive paralytic strains for the ability to bind ³H-saxitoxin, a ligand which specifically blocks sodium channels¹⁻⁴. Three of these 11 strains reproducibly showed reductions in ³H-saxitoxin-binding activity in a preliminary screening. Two of these strains have been analysed in detail.

One of these, the seizure^{ts-2} mutant, was isolated by C. F. Wu and B. Ganetzky. It exhibits reversible paralysis at 39 °C, a temperature at which wild-type flies are not paralysed. To determine if this mutant showed abnormalities in number or affinity of saxitoxin receptors, we examined ³H-saxitoxin binding at 4 °C and 39 °C in membrane extracts prepared from heads of mutant and wild-type strains (Fig. 1). In one experiment at 39 °C, the K_d for saxitoxin binding to seizure^{ts-2} extracts was double that for the wild type (Fig. 1a). This difference in K_d was seen in four experiments with three different pairs of extracts. The mean K_d for binding ($\pm$ s.e.m.) in seizure^{ts-2} extracts was 6.52 ± 0.52 nM compared with 3.74 ± 0.19 in wild-type extracts that were assayed simultaneously. This difference is statistically significant. (Using a *t*-test for matched pairs, $t = 6.48$ and $P = 0.01$.) In the experiment shown in Fig. 1a, there is a slightly lower B_{max} in seizure^{ts-2} extracts than in the wild type. However, when four different experiments are averaged together, the mean number of binding sites is not significantly different for mutant (127 ± 14.3 fmol per mg protein) versus wild type (135.5 ± 7.8 fmol per mg protein) ($t = 0.648$ and $P = 0.56$). The difference in K_d is temperature dependent since it is not seen at 4 °C (Fig. 1b). This temperature-induced change in K_d suggests a structural alteration affecting the saxitoxin receptor. This change appears to be specific for the sodium channel since the binding of α -bungarotoxin to a putative nicotinic cholinergic receptor⁸ in seizure^{ts-2} extracts does not show temperature-dependent changes.

Structural alterations of proteins can cause changes in their pH sensitivity⁹. As indicated in Fig. 2a, the activity of seizure^{ts-2} extracts at 39 °C shows a different pH profile than wild type. Complete saturation curves were constructed for seizure^{ts-2} and wild type at pH 6.07, 6.81 and 7.15 and double reciprocal plots (1/bound against 1/(saxitoxin concentration)) were done to

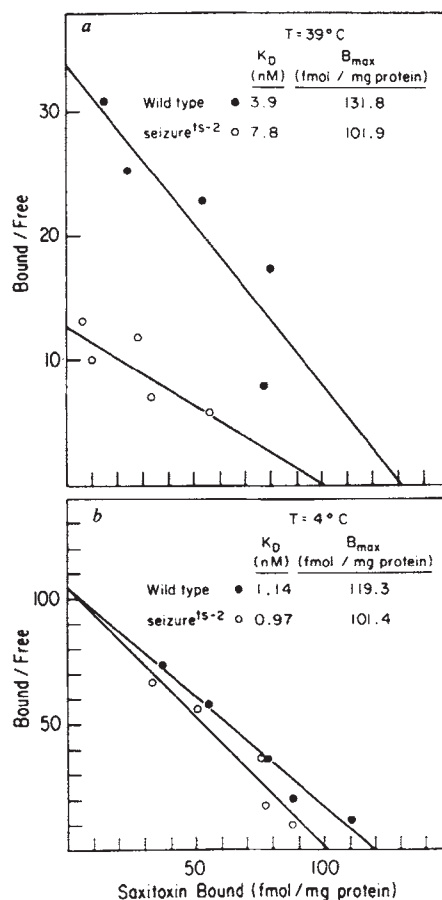


Fig. 1 Effect of temperature on ³H-saxitoxin binding to membrane extracts from wild-type flies and from seizure^{ts-2} mutants. *Drosophila* heads were collected and homogenized, and debris was removed as described previously¹⁸. Crude membranes were prepared by centrifugation at 40,000 *g*_{max} for 40 min and the resulting upper, buffy pellet was resuspended in assay buffer¹⁸ to a concentration equivalent to 200 mg ml⁻¹. The resuspension was done in a glass tissue grinder using 10 strokes of a Teflon pestle driven at 1,700 r.p.m. Membrane aliquots (equivalent to 20 mg heads) were preincubated for 10 min at either 39 °C (a) or 4 °C (b). ³H-saxitoxin (specific activity 18–27 d.p.m. fmol⁻¹) was then added and the samples (150 µl final volume) were incubated for 6 min at the same temperature. Nonspecific binding was determined in the presence of 10 µM tetrodotoxin. At the end of the incubation period, 125 µl of each sample was filtered on a vacuum manifold through pre-wetted Whatman GF/B glass fibre filters and washed for 2–3 s with 10 ml of ice-cold assay buffer. Filters were counted in 8 ml of scintillation cocktail¹⁸. The net binding was calculated by subtracting the nonspecific saxitoxin binding (in the presence of 10 µM tetrodotoxin) from the total binding measured in the absence of tetrodotoxin and then plotted according to Scatchard¹⁹. In this analysis, the x-intercept indicates the number of saturable binding sites (B_{max}) in each extract and the slope of the line is equal to the negative reciprocal of the apparent dissociation constant (K_d).

determine K_d and B_{max} at each pH. We found that the number of saturable-binding sites (B_{max}) in both mutant and wild type remained constant over this pH range. However, the apparent K_d for saxitoxin binding was consistently higher for the mutant than for wild type over the same pH range. The seizure mutation may alter the pK_a of the saxitoxin-binding site.

This dramatic shift in pH profile is consistent with the suggestion that the saxitoxin receptor is structurally altered in seizure^{ts-2} mutants. However, the apparent K_d for binding to this receptor is influenced by electrical charges fixed on the membrane near the binding site^{10,11} and it is possible that differences in the microenvironment of the channel, due perhaps to altered phospholipids, could also account for the observed

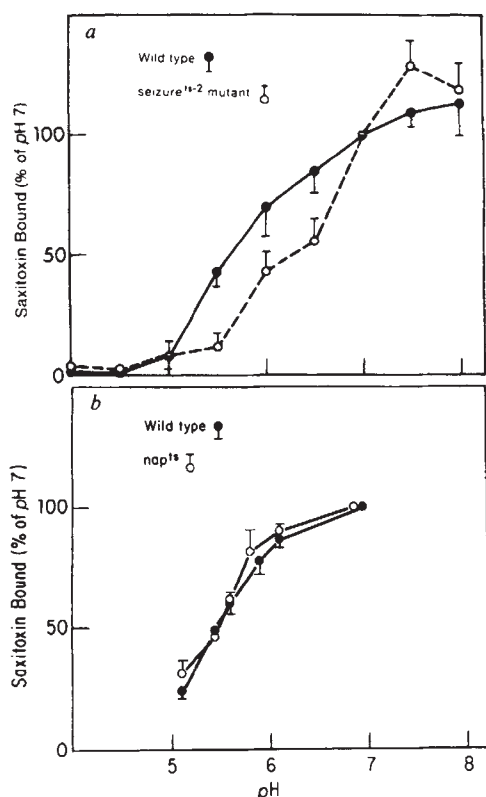


Fig. 2 Comparison of pH sensitivity of ^3H -saxitoxin binding in wild-type, sei^{ts-2} , and nap^{ts} extracts. Membranes were prepared as described in Fig. 1 legend but resuspended at 400 mg heads per ml in distilled water rather than assay buffer. Tetrodotoxin and ^3H -saxitoxin were also diluted in distilled water just before the start of the experiments. The pH of the assay buffer was regulated with a 10 mM final concentration of the following buffers: pH 4.0–5.5, citric acid; pH 5.5–7.0, cacodylic acid; pH 7.0–8.0, HEPES. Membrane aliquots (50 μl) were mixed with 50 μl of threefold concentrated assay buffer of the appropriate pH and preincubated with either 25 μl tetrodotoxin (to give a final concentration of 10 μM) or distilled water. 25 μl ^3H -saxitoxin was added then, and samples were incubated as described below and filtered as described in Fig. 1 legend. The pH of the assay buffer wash was the same as that used for the incubations. The data represent the average ($\pm$ s.e.m.) of three independent experiments. *a*, Membranes were resuspended using 30 strokes rather than 10. Samples were preincubated at 39 $^{\circ}\text{C}$ for 10 min, ^3H -saxitoxin was added to a final concentration of 2–3 nM and samples were incubated for 6 min. *b*, Samples were preincubated at 4 $^{\circ}\text{C}$ for 10 min, ^3H -saxitoxin was added to a final concentration of 2 nM and samples were incubated for 15 min before filtration.

differences in toxin affinity. If the seizure locus does code for a protein component of the voltage-sensitive sodium channel, then sei^{ts-2} might show co-dominance in behavioural and biochemical assays because heterozygotes ($sei^{ts-2}/+$) would have a mixture of wild type and mutant channels. Co-dominance has been demonstrated both behaviourally and biochemically for certain mutations in the structural gene for acetylcholinesterase¹². In contrast, as demonstrated in yeast, mutations reducing activity of enzymes involved in lipid biosynthesis are recessive¹³. As predicted for a mutation in a structural gene for a channel polypeptide, sei^{ts-2} shows co-dominance at the behavioural level. Most heterozygotes ($sei^{ts-2}/+$) become paralysed within 60 s at 40 $^{\circ}\text{C}$, a temperature which has no immediate effects on wild-type flies.

Electrophysiological^{6,14} and preliminary biochemical^{15–17} analyses suggest that another temperature-sensitive paralytic mutation nap^{ts} also affects voltage-sensitive sodium channels. This mutation causes paralysis above a nonpermissive temperature of 34 $^{\circ}\text{C}$ (ref. 5). A Scatchard analysis of saxitoxin-

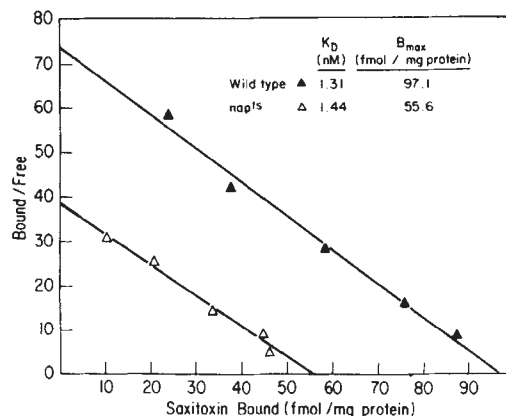


Fig. 3 Scatchard analysis of ^3H -saxitoxin binding to nap^{ts} and wild-type extracts. Extracts were prepared and incubations were conducted as described in Fig. 1 legend except that samples were preincubated for 10 min at 4 $^{\circ}\text{C}$ and then incubated with various concentrations of ^3H -saxitoxin for 15 min at 35 $^{\circ}\text{C}$ before filtration.

binding to nap^{ts} and wild-type extracts at 35 $^{\circ}\text{C}$ shows that the number of binding sites is reduced by about 40% in the mutant relative to the wild type (Fig. 3). In several experiments, this reduction varies from 30 to 50% and does not depend on incubation temperature, in the range 4–38 $^{\circ}\text{C}$. The nap^{ts} mutation has no effect on the apparent dissociation constant of the remaining binding sites (Fig. 3). Furthermore, the pH sensitivity of saxitoxin binding to nap^{ts} extracts is normal (Fig. 2b).

It appears that nap^{ts} affects sodium channel levels without altering activities for other neuronal markers. α -Bungarotoxin binding, a synaptic marker, is normal in nap^{ts} mutant extracts¹⁶, as is another neuronal marker, ($\text{Na}^+ - \text{K}^+$)ATPase. Wild-type flies and nap^{ts} mutants have maximum velocities (V_{max}) of enzyme activity of 0.364 μmol per min per mg protein and 0.343 μmol per min per mg protein, respectively. The wild-type enzyme has a K_m of 0.50 mM compared with 0.45 mM in the nap^{ts} mutant.

It has been suggested that the nap locus codes for a component of the voltage-sensitive sodium channel¹⁷. If this were true, heterozygotes ($nap^{ts}/+$) should show numbers of saxitoxin-binding sites intermediate between the mutant (nap^{ts}/nap^{ts}) and the wild-type ($+/+$). A reduction in the number of saxitoxin-binding sites would be more pronounced in flies that carry two copies of the mutant allele and one copy of the wild type ($+/nap^{ts}/nap^{ts}$). We constructed such a strain using a Y chromosome containing a duplication of a small region of the second chromosome (41–43 A of the salivary chromosome map) which includes the nap^{ts} locus⁵. The number of saturable saxitoxin-binding sites in this strain at 4 $^{\circ}\text{C}$ ($B_{max} = 117.9 \pm 0.6$ fmol per mg protein) was indistinguishable from that of wild type (see Fig. 1b) and was significantly higher than that of homozygous mutants (nap^{ts}/nap^{ts}) of similar genetic background ($B_{max} = 87.6 \pm 3.8$ fmol per mg protein). This lack of co-dominance makes it unlikely that the nap locus encodes a channel subunit. However, the nap^{ts} mutation may result in structural alterations (such as post-translational modifications) which affect the efficacy with which channels are incorporated into membranes. Flies heterozygous for a mutation affecting a modifying enzyme would be normal if 50% of that enzyme activity were sufficient for complete modification.

We wanted to determine whether the temperature-induced paralysis in the nap^{ts} stock was due simply to a reduced number of membrane-bound sodium channels. If this were true, a pharmacological reduction in the number of functional sodium channels in wild-type flies should mimic (phenocopy) the phenotype of temperature-induced paralysis. As shown in Fig. 4, wild-type flies fed a sublethal dose of tetrodotoxin became reversibly paralysed at a temperature at which buffer-fed control wild-type flies were unaffected. The kinetics of paralysis and

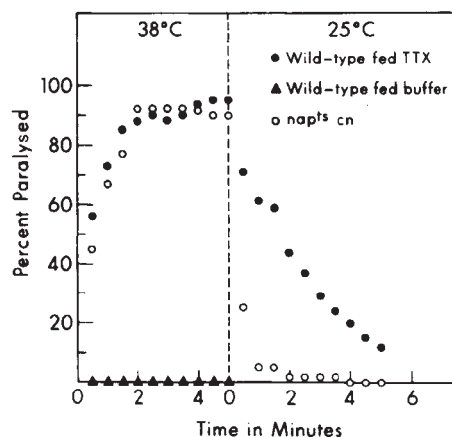


Fig. 4 Effects of temperature on wild-type flies fed sublethal doses of tetrodotoxin. Canton-S wild-type flies (3–6 days post-eclosion) were placed in vials containing two 2.5-cm filter paper circles wet with 150 μ l 20 mM citrate buffer (pH 4.8) alone (controls) or with buffer containing 10 μ g ml⁻¹ (31.4 μ M) tetrodotoxin (TTX). After 24 h at 21 °C this dose of TTX caused 75–90% lethality. Survivors were selected that maintained good locomotor activity and could still climb the walls of the glass culture vial. Control and treated flies were transferred to clean vials and exposed to 38 °C for 5 min. The number of flies paralysed was recorded at 30-s intervals. After 5 min, flies were transferred to 25 °C and the rate of recovery from paralysis was recorded. The paralysis of *nap^{ts}* mutants (genetically marked with an eye colour mutation *cn*) was determined simultaneously as a control.

recovery of the *nap^{ts}* mutant are shown for comparison. Although toxin-fed wild-type flies became paralysed with kinetics similar to the *nap^{ts}* mutant, the recovery from paralysis was slower in the toxin-fed wild-type than in the mutant. Reasons for this are unknown. Nevertheless, a reduction in number of functional channels is sufficient to produce a temperature-induced paralytic phenotype.

Using ligand binding, we have identified a subset of temperature-sensitive paralytic mutants with abnormal saxitoxin-binding phenotypes. Mutations affecting the saxitoxin-binding site will be useful for determining the genetic relationship between this and other distinct pharmacological sites of the sodium channel. Such mutants can also be used to study the developmental regulation of sodium channels, and the general role of cell excitability in development.

We thank Nancy Martinez, Charlene Comastri, Gene Belknap and John Yee for technical assistance, Drs Martin Chalfie, Barry Ganetzky, Jeffrey C. Hall and R. Scott Hawley for critical comments on this manuscript, Barry Ganetzky and Shankar J. Kulkarni for providing copies of temperature-sensitive paralytic mutant stocks before their data were published on these mutants. This work was supported by NIH grants NS 16204 to L.M.H. and NS 18467 to G.R.S. L.M.H. is an Irma T. Hirsch-Monique Weill-Caulier Career Scientist Awardee.

Received 15 April; accepted 8 December 1983.

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Evidence for immunological cross-reaction between sporozoites and blood stages of a human malaria parasite

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Malaria parasites (*Plasmodium* spp.) show a complex pattern of development in the mammalian host and many studies support the view that the surface of the sporozoite, injected by the mosquito, has no antigens in common with the erythrocytic stage of development¹. For example, immunization with the erythrocytic parasites generates antisera with negligible titre by indirect immunofluorescence to the sporozoite surface^{2–4}. Although monoclonal antibodies prepared against erythrocytic stages were reported to show cross-reaction to the sporozoite stage⁵, this appeared to be due to cytoplasmic antigens exposed by the method of sporozoite preparation^{3,5}, and in *Plasmodium knowlesi*, a cDNA clone coding for the circumsporozoite antigen, the major protein of the sporozoite surface, showed no hybridization to mRNA isolated from the erythrocytic stages⁶. Here, however, we present evidence for an antigenic determinant shared by the sporozoite surface and the erythrocytic stages of the human malaria parasite, *P. falciparum*. Moreover, our studies show that the antigen(s) elicit a strong immune response in man.

Monoclonal antibodies against the cultured erythrocytic stages of *P. falciparum* K1, a Thai isolate⁷, were tested by indirect immunofluorescence microscopy against *P. falciparum* sporozoites. One of the 22 tested, McAb 5.1, gave a strongly positive reaction to the sporozoite preparation (Fig. 1a). The titre of the McAb 5.1 ascites fluid was very high (1 in 10⁶ dilution) and equally active on sporozoites and erythrocytic stages. The other monoclonal antibodies tested did not stain sporozoites at a dilution of 1:100 (Fig. 1b).

Both air-dried and glutaraldehyde-fixed sporozoites are stained by indirect immunofluorescence with McAb 5.1. The latter method of preparation only allows antibody to bind to surface determinants³ and the clear microscope image it provides is strong evidence that the antigen is located at the sporozoite surface. These results should be compared with indirect immunofluorescence of the erythrocytic parasites. Here the 5.1 epitope appears to be associated with the parasitophorous vacuole and parasite-derived inclusions in the infected red blood cell (ref. 7 and Fig. 1c). Another monoclonal antibody, 7.7, gives an identical immunofluorescence pattern⁷ to McAb 5.1 in infected red blood cells but does not react with the sporozoite stage (data not shown). Different immunofluorescence patterns are obtained on infected red blood cells with other monoclonal antibodies, for example McAb 7.3⁷ (Fig. 1d).

The 5.1 antigen can be clearly detected in SDS extracts of parasites prepared by saponin lysis of infected red blood cells. The proteins were fractionated by SDS-polyacrylamide gel electrophoresis (PAGE) (Fig. 2, lanes 1, 2), transferred to nitrocellulose, probed with McAb 5.1 and the antigen visualized by enzyme-linked immunosorbent assay (ELISA)⁸ (Fig. 2, lane 4). A single band carries the 5.1 epitope. It migrates as a protein of molecular weight (*M_r*) 23,000. The antigen can be partially

purified from Nonidet P-40 (NP40) extracts of parasitized erythrocytes on a McAb 5.1-Sepharose column, despite low affinity of the antibody for its antigen (which complicated earlier studies⁷). This preparation (Fig. 2, lanes 3, 5) also shows a single band carrying the epitope migrating slightly faster (M_r 22,000) than that in the unpurified extracts, perhaps because the protein is cleaved during purification.

The 5.1 antigen is parasite-encoded. NP40 extracts from parasites metabolically labelled with ³⁵S-methionine were analysed by affinity chromatography and gel electrophoresis. The separated proteins were visualized by autoradiography (Fig. 2, lanes 6, 7). A unique, labelled protein band (M_r 22,000) is retained by the McAb 5.1 affinity column (lane 7).

We now present evidence that the 5.1 epitope is determined by the primary amino acid sequence of the antigen molecule. *P. falciparum* mRNA⁹ can be translated in rabbit reticulocyte extracts¹⁰ into proteins which are not processed or modified. The McAb 5.1-Sepharose column traps several of the *in vitro* products nonspecifically (Fig. 2, lane 9). These are also trapped by other monoclonal antibody-Sepharose columns (see lane 10). However, the McAb 5.1 column retains a unique protein, M_r 24,500 (lane 9). No other column (3/3 tested) retains this protein (see lane 10). It is reasonable to conclude that it carries the 5.1 epitope in the amino acid sequence. Note that the primary transcript appears larger than the mature antigen, suggesting that it is processed or modified *in vivo*.

These results lead to the important conclusion that the 5.1 epitope of the sporozoite and erythrocytic stages is not a common modification of two entirely different proteins, but the same amino acid sequence(s) found in two proteins made at different stages of the life cycle.

The 5.1 antigen is recognized by human sera from areas (Nigeria or Gambia) endemic for *P. falciparum* (Fig. 3). Total parasite proteins and proteins immunoadsorbed on McAb 5.1-Sepharose were subjected to SDS-PAGE (Fig. 3a). They were transferred to nitrocellulose and probed by ELISA. For comparison we studied proteins immunoadsorbed by McAb 7.7 which gives the same immunofluorescence pattern as McAb 5.1. Shown in Fig. 3, the following antibody probes were used: b, a mixture of monoclonal antibodies 5.1 and 7.7; c, sera (pooled) from individuals living in an area endemic for *P. falciparum* malaria; and d, serum from an individual never exposed to *P. falciparum*.

The bands revealed by endemic sera (Fig. 3c) and monoclonal antibodies 5.1 and 7.7 (Fig. 3b) are specific. No bands are seen with the nonimmune serum (Fig. 3d) or with other monoclonal antibody controls (data not shown). Figure 3b shows the 5.1 antigen (M_r 22,000, 22K). It is quite distinct from the 7.7 antigen (33K). Both appear together in the total parasite protein (Fig. 3b, lane 1) and separately in the purified antigen preparations (lanes 2, 3). The pooled human sera (Fig. 3c) have no detectable antibodies against the purified 7.7 antigen (Fig. 3c, lane 3). By contrast, the purified 5.1 antigen is very strongly recognized (lane 2). Note that the pooled human sera detect many antigens amongst the total parasite proteins (lane 1) but there is one particularly strong band (arrow). A comparison of antigen band positions and intensities in tracks 1 and 2 of Fig. 3b and c suggests that this is the 5.1 antigen. In fact, Sepharose-bound 5.1 antigen reacts with as much as 1% of the antibodies in these pooled sera (data not shown).

We conclude that a major immunogenic protein of the erythrocytic stage of *P. falciparum* contains an antigenic determinant also present in a protein at the surface of sporozoites. In this connection we were interested to learn that Coppel and co-workers¹¹ have identified a 220K protein of the erythrocytic stages with an immunofluorescence pattern similar, if not identical, to that of the 5.1 antigen. Remarkably, it has random repeats of an 11 amino acid sequence. This repetitive feature has also been described in the major circumsporozoite protein of *P. knowlesi*⁶.

The blood stage antigen bearing the 5.1 epitope appears highly immunogenic; why then has immune cross-reaction to the

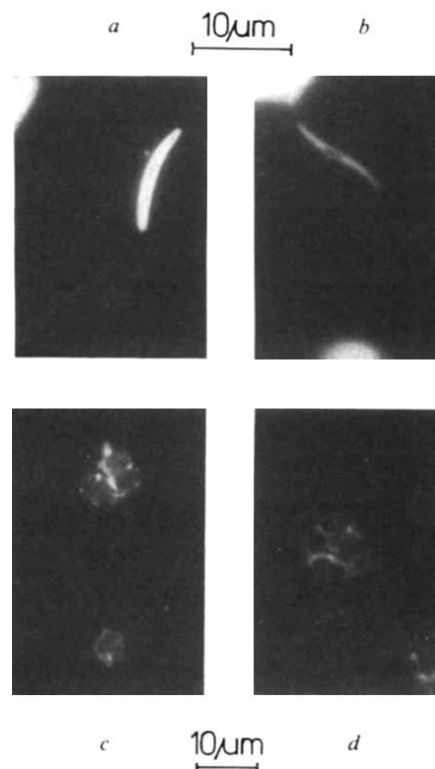


Fig. 1 Indirect immunofluorescence staining of *P. falciparum* microscope preparations using monoclonal antibodies. a, b, Glutaraldehyde-fixed sporozoites collected in Thailand (a gift of Dr Ruth Nussenzweig). c, d, Air-dried thin blood smears of an asynchronous *in vitro* culture¹³ of *P. falciparum* (isolate KI from Thailand¹⁴). The parasitaemia was 5%.

Methods: All slides were fixed in acetone for 5 min. The staining was carried out at room temperature in a moist chamber as detailed in ref. 7. The slides were incubated with monoclonal antibodies for 30 min, stained with fluorescein isothiocyanate-conjugated polyvalent rabbit anti-mouse immunoglobulin (Miles-Yeda) and counterstained with Evans blue (0.1% in phosphate-buffered saline (PBS)). The slides were examined by fluorescence microscopy after mounting in 50% glycerol. a, c, Monoclonal antibody 5.1 was used in the primary incubation; b, d, for comparison, the staining produced by a different monoclonal antibody called 7.3 (see ref. 7). Bound antibody appears as a vivid green fluorescence (a, c, d) whereas parasites which do not bind antibody (b) appear red. Photographs were taken at $\times 1,000$ magnification using Kodak Tri-X Pan film.

sporozoite surface not been detected experimentally? One explanation, with important implications, is that the 5.1 antigen becomes strongly recognized by human sera only through long-term continual exposure to the antigen, as experienced by individuals living in areas endemic for malaria. Experimental tests for stage cross-reactivity of monoclonal antibodies or antisera, raised by short-term immunization protocols, would not reveal this epitope.

It is interesting that McAb 7.7 recognizes an epitope on a totally different molecule from McAb 5.1 despite the fact that they cannot be distinguished by immunofluorescence microscopy. It is particularly striking that the 22K protein of McAb 5.1 is very immunogenic in man whilst the 33K protein of McAb 7.7 elicits no detectable immune response. It is also relevant that, unlike the 7.7 epitope, that of 5.1 is not present in all strains of *P. falciparum*⁷.

Natural immunity to malaria of individuals living in endemic areas is only fully acquired by adolescence¹². An antigen to which an immune response is only raised very slowly therefore may be of importance in the development of immunity. Such an antigen would be of particular importance if it were present on both the sporozoite and erythrocytic stages of the parasite.

Fig. 2 Characterization of 5.1 antigen. Proteins were separated by SDS-PAGE¹⁵ on 10% gels. Lane 1 contains total parasite protein stained with Coomassie brilliant blue R. Lanes 2 and 3 contain, respectively, total parasite protein and partially purified 5.1 antigen visualized by silver staining¹⁶. The position of 5.1 antigen is revealed in lanes 4 and 5 by Western blotting⁸ of total parasite proteins (lane 4) and partially purified antigen (lane 5) and detection by an ELISA procedure⁸. Lanes 6–10 show autoradiographed, ³⁵S-methionine-labelled proteins. Total parasite proteins (lane 6) and 5.1 antigen (lane 7) were metabolically labelled in culture. Lane 8 shows total parasite proteins synthesized by *in vitro* translation of *P. falciparum* mRNA. From the total *in vitro* translation products 5.1 antigen (lane 9) has been partially purified on a 5.1 monoclonal antibody affinity column. For comparison, lane 10 shows the unrelated antigen, P190, recognized by McAb 7.3 (ref. 7), prepared in a similar way. Specifically adsorbed bands in the two lanes are arrowed.

Methods: The proteins in lanes 1 and 2 were prepared from an asynchronous *in vitro* culture¹³ of *P. falciparum* (5×10^9 erythrocytes; 5% parasitaemia), washed once in PBS and lysed in 0.1% saponin in PBS at 4°C. Released parasites were washed three times in PBS before pelleting and freezing at -70°C until required. For electrophoresis, the pellet was solubilized by boiling in gel sample buffer. Lane 2 was deliberately overloaded to allow detection of the antigen after Western blotting (see lane 4 below), since the 5.1 antigen constitutes a minor proportion of the total proteins (compare lanes 2 and 3 with 4 and 5). 5.1 antigen (lane 3) was prepared by affinity chromatography. An asynchronous parasite protein extract, solubilized with NP40 (BDH)¹⁷, was applied to a 5.1 monoclonal antibody affinity column. This was prepared by binding McAb 5.1 (purified from ascites fluid by (NH₄)₂ SO₄ precipitation and DEAE ion-exchange chromatography) to CNBr-activated Sepharose 4B (Pharmacia). After applying the extract the column was washed briefly in the extraction buffer¹⁷, before elution of bound proteins in 8 M urea (4°C). Proteins of lanes 2 and 3 were transferred to nitrocellulose paper (lanes 4, 5) by Western blotting⁸ and the position of 5.1 antigen was revealed by ELISA⁸. The nitrocellulose blot was first probed with McAb 5.1 (ascites fluid at a 1 in 200 dilution), then a polyvalent rabbit anti-mouse IgG (serum at a 1:500 dilution) and finally with horseradish peroxidase-conjugated goat anti-rabbit IgG (Sigma) (at a 1:1,000 dilution). The conjugated enzyme was reacted with *o*-dianisidine dihydrochloride (Sigma) and hydrogen peroxide, giving a brown colour at the position of 5.1 antigen. The parasite proteins in lanes 6 and 7 were labelled metabolically with ³⁵S-methionine in an asynchronous culture¹³ of *P. falciparum* by the method of Deans *et al.*¹⁸. Proteins of a total NP40 extract (see above) of the labelled culture appear in lane 6 (3,000 c.p.m. of trichloroacetic acid (TCA)-precipitable material). Lane 7 shows protein purified by the McAb 5.1 affinity column (see above) from the labelled parasite extract (3×10^6 c.p.m. of TCA-precipitable material). The parasite proteins in lanes 8, 9 and 10 were synthesized in a reticulocyte lysate *in vitro* translation system¹⁰ supplemented with ³⁵S-methionine and *P. falciparum* poly(A)⁺ mRNA from asynchronous cultures⁹. Lane 8 shows total *in vitro* translation products (5,000 c.p.m. of TCA-precipitable material). Lane 9 shows proteins adsorbed from the total *in vitro* translation products (5×10^6 c.p.m. of TCA-precipitable material) on a McAb 5.1 affinity column (see above). Lane 10 shows the translation products adsorbed in the same way, by an affinity column coupled with a different monoclonal antibody, McAb 7.3 (ref. 7). The gel was processed for fluorography¹⁹, dried and autoradiographed (lanes 6 and 10 for 3 weeks and lanes 7, 8 and 9 for 1 week). Molecular weight markers (Pharmacia): ferritin (220,000), phosphorylase *b* (94,000), albumin (67,000), catalase (60,000), ovalbumin (43,000), lactate dehydrogenase (36,000), carbonic anhydrase (30,000), trypsin inhibitor (20,100), ferritin (18,500) and α -lactalbumin (14,400).

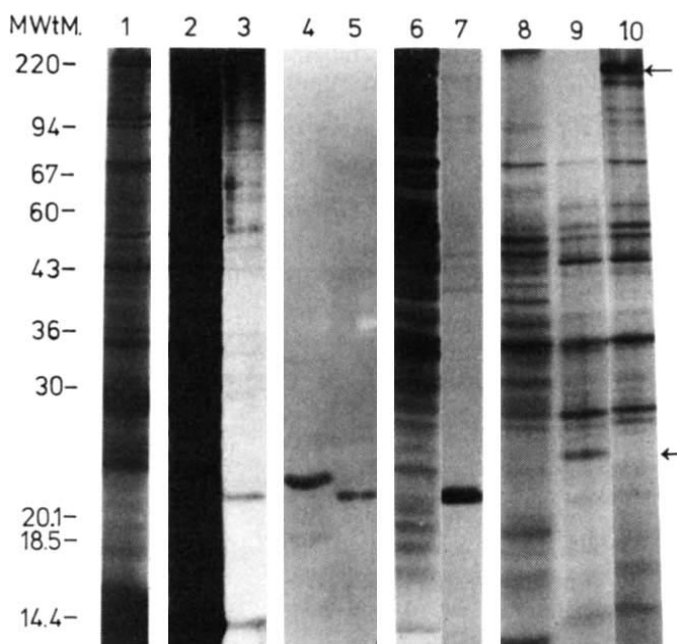
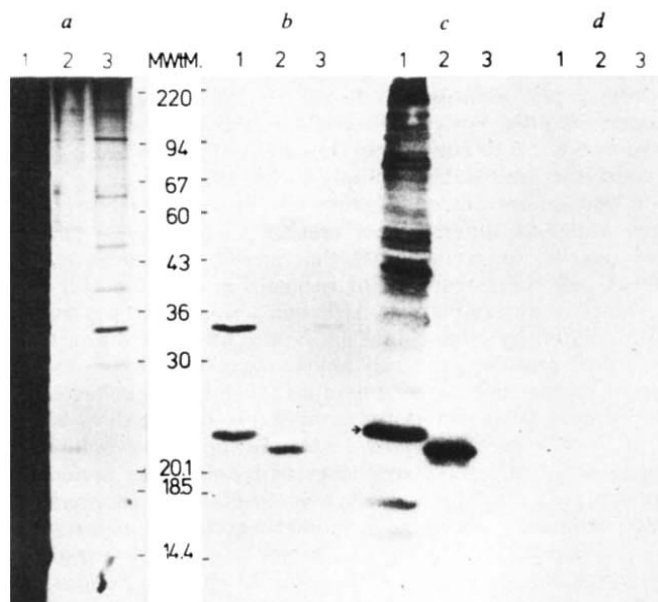


Fig. 3 Recognition of 5.1 antigen by human sera. In all lanes proteins were separated by SDS-PAGE¹⁵ on 10% gels. In *a*, the proteins were visualized by silver-staining¹⁶. Lane 1 is total parasite protein (see Fig. 2). This lane is overloaded to enable antigens to be seen after Western blotting (see below). Lane 2 is partially purified 5.1 antigen (see Fig. 2). Lane 3 is partially purified 7.7 antigen prepared by affinity chromatography using a 7.7 monoclonal antibody affinity column. (Methods in Fig. 2.) *b*, *c*, *d*. The proteins of *a* transferred to nitrocellulose by the Western blotting procedure⁸. These blots were then probed with antibody by the ELISA procedure, as described for Fig. 2. Blot *d* was initially probed with human serum (1:200 dilution) from an individual never exposed to *P. falciparum* malaria. Blot *c* was initially probed with human sera (1:200 dilution) obtained by pooling 50 serum samples from pregnant women living in Nigeria, an area endemic for malaria. Both blots *c* and *d* were then probed with horseradish peroxidase-conjugated goat anti-human IgG (Sigma) (1:1,000 dilution). Blot *b* was probed first with the monoclonal antibodies 5.1 and 7.7 (1:200 dilution) and then probed using polyvalent rabbit anti-mouse IgG (1:500 dilution) followed by horseradish peroxidase-conjugated goat anti-rabbit IgG (1:1,000 dilution). For all blots *b*, *c* and *d*, the position of bound antibody was determined using the enzymic colour reaction as detailed in Fig. 2. Molecular weight markers are as in Fig. 2.



Our studies raise two major questions: are the antibodies against the 5.1 antigen in endemic sera protective, and, do the majority of them cross-react with sporozoites? Since we are now able to extract significant amounts of the 5.1 antigen from erythrocytic parasites, it should be possible to investigate these questions.

We thank Drs E. Nardin and R. Nussenzeig for gifts of sporozoites, support and advice, Dr A. Osland for help and advice, Dr J. McBride for monoclonal antibodies, Professor Sir Ian McGregor and Mrs E. Williams for endemic sera, Mrs J. King for technical assistance, Dr P.-L. Yap and the Edinburgh Blood Transfusion Service for continued support and the MRC.

Received 6 September; accepted 13 December 1983.

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Lymphocyte suppression in leprosy induced by unique *M. leprae* glycolipid

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Leprosy remains a significant medical and social problem in many developing countries. The varied forms of the disease form a spectrum¹. At one pole, tuberculoid leprosy, patients develop high levels of cell-mediated immunity which results in the killing and clearing of bacilli in the tissues. At the lepromatous pole, patients exhibit a selective immunological unresponsiveness to antigens of *Mycobacterium leprae* so that the organisms inexorably multiply in the skin. We have suggested that in lepromatous leprosy one or a small number of unique antigenic determinants present on *M. leprae* might induce specific suppressor cells that inhibit the reactivity of helper T-cell clones capable of recognizing other specific or cross reactive determinants². Although unique epitopes have been identified by monoclonal antibodies on a small number of *M. leprae* proteins³, the only unique species of antigen present in *M. leprae*, and not on any other species of mycobacteria so far examined, is a phenolic glycolipid (gly-I)⁴. We show here that this unique antigen of *M. leprae* is capable of inducing suppression of mitogenic responses of lepromatous patients' lymphocytes *in vitro* and provide evidence that the suppressor T cells recognize the specific terminal trisaccharide moiety.

It remains unclear why the vast majority of people exposed to *M. leprae* develop no clinical disease, and why only a minority who do develop clinical disease become immunologically unresponsive to antigens of the organisms. Lepromatous patients who are anergic to antigens of *M. leprae* frequently show cell-

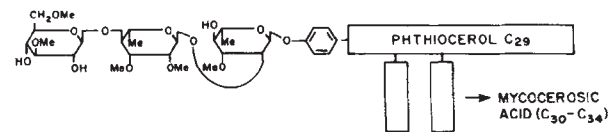


Fig. 1 Schematic structure of *M. leprae* phenolic gly-I. It is composed of 3,6-di-O-methylglucose, 2,3-di-O-methylrhamnose, 3-O-methylrhamnose linked to phenol-dimycocerosyl phthiocerol.

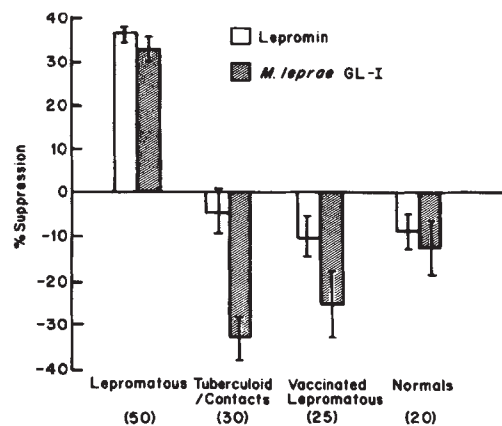


Fig. 2 Suppression of mitogenic responses of peripheral blood lymphocytes of leprosy patients and normals to Con A in the presence of *M. leprae* specific phenolic gly-I and Dharwendra lepromin. Peripheral blood mononuclear cells were obtained from heparinized blood by Ficoll-Hypaque centrifugation. The suppression of mitogenic response of lymphocytes to Con A, induced by lepromin and phenolic glycolipid, was assayed as described earlier². Briefly, 2×10^5 lymphocytes were cultured in triplicate in RPMI 1640 containing 10% heat inactivated pooled human AB serum with (1) no additions, (2) $2.5 \mu\text{g ml}^{-1}$ Con A, (3) Con A plus Dharwendra lepromin (1:10), $0.5 \mu\text{g ml}^{-1}$ *M. leprae* phenolic gly-I in the form of liposomes or control liposomes. Liposomes were prepared by the method of Six *et al.*¹². A mixture containing 2.0 mg sphingomyelin, 0.73 mg cholesterol, 0.065 mg dicetylphosphate and 0.23 mg *M. leprae* gly-I in 125 μl of Tris-NaCl buffer (pH 8.0) was sonicated for 1 h with glass beads. The cultures were labelled on day 2 with $1 \mu\text{Ci } ^3\text{H}$ -thymidine (specific activity 5 Ci mmol⁻¹) per well and harvested 18 h later. The data were analysed by analysis of variance and Duncan's multiple range test. Significance was accepted at the $P < 0.05$ level. Numbers in parenthesis represent the number of subjects studied in each group.

mediated immunity to antigens of the tubercle bacillus although all known species of protein and glycoprotein antigens in *M. leprae* are either serologically identical or cross-reactive with those of BCG⁵. Some experimental support *in vitro* for the existence of lepromin-induced suppressor activity of mononuclear cells from lepromatous leprosy patients has appeared. Adherent cells from lepromatous patients, presumably monocytes, have been found to suppress mitogen and antigen responses *in vitro*⁵⁻⁸. Lepromin-induced suppressor T cells have also been implicated in some circumstances. We have previously reported that 84% of patients with lepromatous leprosy have a T cell bearing the OKT5/OKT8 phenotype which can be induced by lepromin to suppress responses of the patients or normal donors lymphocytes to the mitogen, concanavalin A (Con A)⁶. These suppressor cells (T_S) are active in patients with lepromatous and borderline leprosy, but not tuberculoid leprosy or in normal donors. A high percentage (approximately 50%) of the T8 cells from lepromatous patients expressed the activation markers, Fc IgG receptors and HLA-DR antigens⁹. Depletion of these T8 cells in a third of the patients with lepromatous leprosy permitted the remaining T cells to respond vigorously to lepromin, indicating that, in some patients, specifically reactive T cells were present, but unable to respond in the presence of the T suppressor cells.

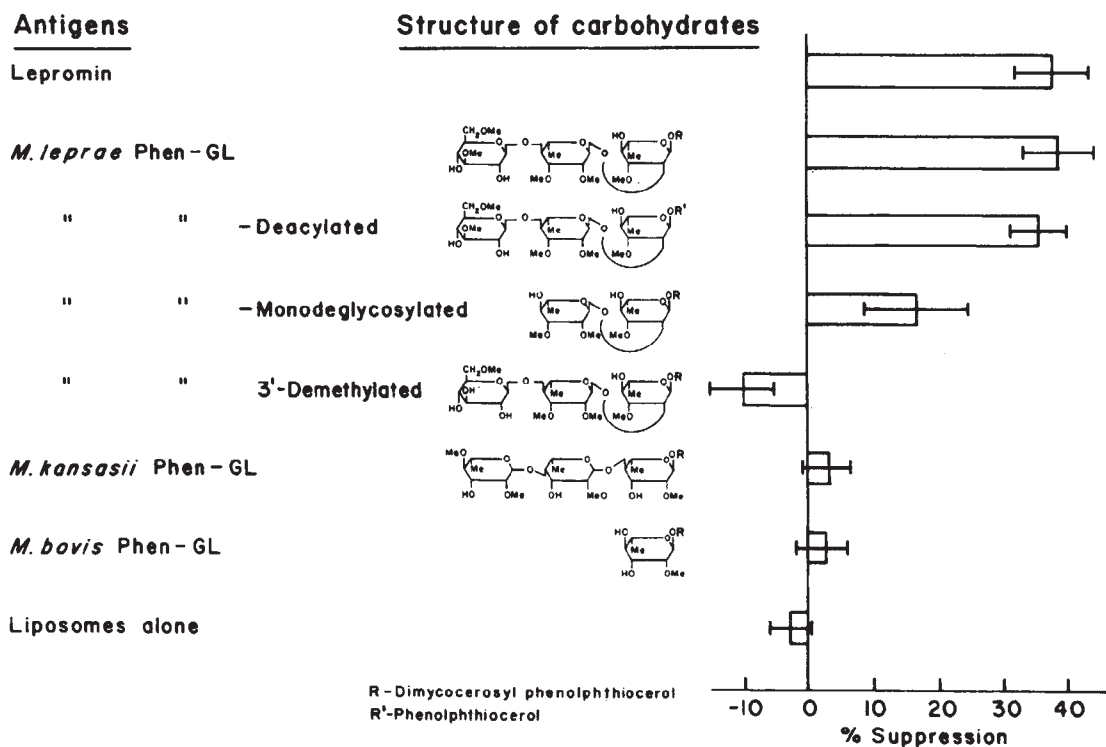


Fig. 3 Suppression of Con A responses of peripheral blood lymphocytes of lepromatous leprosy patients by modified *M. leprae* glycolipid and phenolic glycolipids from other mycobacteria. All the preparations were added in liposomes to the cultures at a conc. of $0.5 \mu\text{g ml}^{-1}$, except deacylated GL-I, that was suspended in PBS and added at the same concentration. The chemical nature of the lipids tested are as follows: *M. leprae* phenolic gly-I: 3,5-di-O-Me- β -D-Glcp(1-4)2,3-di-O-Me- α -L-Rhap(1-2)3-O-Me- α -L-Rhap-R; *M. leprae* phenolic gly-I (monodeglycosylated): 2,3-di-O-Me- α -L-Rhap(1-2)3-O-Me- α -L-Rhap-R; *M. leprae* phenolic glycolipid-III (3'-demethylated): 6-O-Me- β -D-Glcp(1-4)2,3-di-O-Me- α -L-Rhap(1-3)3-O-Me- α -L-Rhap-R; *M. kansasii* phenolic glycolipid (mycoside A): 2,4-di-O-Me-L-Rhap(1-4)2-O-Me-L-Fucp(1-4)2-O-Me-L-Rhap-R (tentative structure); *M. bovis* phenolic glycolipid (mycoside B): 2-O-Me- α -L-Rhap-R; Glcp: glucopyranose; Rhap: rhamnopyranose; Fucp: fucopyranose; R: dimycocerosyl phenolphthiocerol.

Since the phenolic glycolipid I, the structure of which is schematically represented below (Fig. 1), is the only unique species of antigen found in *M. leprae*, we explored the possibility that it might be recognized by suppressor cells from lepromatous patients. Phenolic gly-I was prepared from armadillo-derived *M. leprae* as described previously⁴. Because of its insolubility in aqueous medium, it was presented to the lymphocytes from patients with leprosy in the form of liposomes (Fig. 2) or in the more water-soluble deacylated form. Lymphocytes were obtained from lepromatous patients, either untreated or treated for less than three months, whose stage of disease was classified according to the Ridley and Jopling scale¹⁰. The mononuclear cells were cultured for three days with Con A in the absence or presence of Dharmendra lepromin or glycolipid, and suppression of ^3H -thymidine incorporation was measured as described previously². As shown in Fig. 2, significant and comparable suppression of lymphocyte proliferation to Con A was induced by gly-I and lepromin in lymphocytes from lepromatous patients but not from patients with tuberculoid leprosy, lepromin-positive contacts or normal donors. The optimal dose of the glycolipid for inducing suppression in liposomes was found to be $0.5 \mu\text{g ml}^{-1}$. As reported previously⁶, depletion of the OKT8 cells removed the *in vitro* suppression observed by both lepromin and the glycolipid. The significance of the weak stimulation of ^3H -TdR incorporation by gly-I-containing liposomes in lymphocytes from lepromin-positive contacts and tuberculoid patients remains unclear, although preliminary data indicate that depletion of OKT4 cells does not result in a diminution of the ^3H -TdR incorporation and that gly-I fails to induce higher stimulation at 6 days.

To elucidate the specificity of recognition of this unusual antigen by the T_S cells, the ability of a series of modified *M. leprae* glycolipids and structurally different glycolipids prepared from other mycobacteria to induce suppression was examined. The results shown in Fig. 3 indicate that: (1) removal of the

Table 1 Inhibition of phenolic glycolipid and lepromin-induced suppression of Con A responses of peripheral blood lymphocytes of lepromatous leprosy patients by monoclonal antibodies

Monoclonal antibodies to <i>M. leprae</i>	Specificity	% Suppression of Con A response by	
		Phen-GL-I	Lepromin
—	—	42.9 $\pm$ 3.9	38.1 $\pm$ 2.6
46.7	Phen-GL-I terminal disaccharide	—18.9 $\pm$ 7.2	17.8 $\pm$ 6.6
Y ₁	Specific epitope on 68K protein	45.4 $\pm$ 3.8	27.4 $\pm$ 3.5
46.7 + Y ₁	Cross-reactive with surface antigen on <i>M. leprae</i> and other mycobacteria	—23.0 $\pm$ 6.7	16.6 $\pm$ 12.5
D ₁₇		35.3 $\pm$ 4.9	41.8 $\pm$ 5.4
J ₁₂	Dharmendra lepromin	45.5 $\pm$ 4.2	47.9 $\pm$ 5.3

Monoclonal antibodies were produced by the fusion of spleen cells from BALB/c mice immunized with *M. leprae* to myeloma X63 Ag 8.653 cells. The reactivity and specificity of monoclonal antibodies, all of which are IgG₁ was determined by enzyme-linked immunosorbent assay (ELISA) of 18 mycobacterial species (in preparation). In ELISA, mAb 46.7 showed an optical density of 2.23 with gly-I, 0.19 with gly-I lacking the terminal sugar, and 0.01 with gly-I lacking the terminal disaccharide. Suppression was measured as before in the presence or absence of 20 μl per well of culture supernatants from the hybrids making the monoclonal antibodies with the above mentioned reactivities.

mycocerosic acid side chain (deacylated phenolic glycolipid-I) had no effect on *in vitro* suppression; (2) absence of the 3' terminal methyl group (phenolic glycolipid-III)¹¹ abolished the suppression; and (3) removal of the terminal sugar significantly reduced the suppression. No suppression was induced by the comparable glycolipids from *M. kansasii* and *M. bovis* or by

liposomes alone. These data indicate that the suppressor cell has rather exquisite specificity for the terminal trisaccharide found in *M. leprae* gly-I. This was confirmed by testing a series of monoclonal antibodies for their ability to inhibit suppression induced by lepromin or the phenolic glycolipid (Table 1). Monoclonal antibody specific for the terminal disaccharide of the *M. leprae* glycolipid completely inhibited the suppression induced by gly-I, and significantly reduced that by lepromin. A monoclonal antibody to an *M. leprae* specific epitope on a polypeptide of molecular weight 68,000 weakly diminished suppression of the intact lepromin, but was without effect on the purified glycolipid. Monoclonal antibodies to a cross-reactive mycobacterial surface antigen, and to an antigen present in Dharmendra lepromin were also without effect. These results indicate that the phenolic gly-I is at least one of the major suppression-inducing determinants of *M. leprae* in this assay.

It remains to be ascertained whether the phenolic glycolipid and T cells capable of suppressing mitogenic responses are effective in suppressing specific T-cell responses to *M. leprae* antigens in appropriate patients. Nevertheless, it has been possible to associate the lepromin-induced *in vitro* suppressor activity described here with the course of disease by examining a small number of patients with lepromatous leprosy who have been successfully vaccinated with a mixture of killed *M. leprae* and live BCG¹³. As shown in Fig. 2, there is an almost total reduction in both lepromin-induced and gly-I-induced suppression in patients showing clinical improvement following immunotherapy. These results suggest that T_S cells assayed here and the unique phenolic glycolipid of *M. leprae* may be relevant to the clinical unresponsiveness of patients with lepromatous leprosy.

If the selective unresponsiveness of lepromatous leprosy patients is due to the action of suppressor cells induced by the phenolic glycolipid or other antigens, it would appear that successful immunization, either with *M. leprae* plus live BCG or with presumably cross-reactive culturable mycobacteria can overcome this unresponsiveness and engender positive skin test reactivity, histological upgrading of the quality of the lesions and more rapid clearing of antigens. The recent report by Abebe *et al.*¹⁴ indicating that the unresponsiveness to *M. leprae* antigens could be partially overcome *in vitro* by the addition of T-cell conditioned medium containing interleukin-2 (IL-2) would suggest that the mechanism of action of such suppressor cells would be to block either the production of, or responsiveness to, lymphokines by lepromin-responsive T_H cells. Even so, the factors governing the development of T_S cells and the lepromatous form of the disease remain unclear: genetic constitution, defective antigen presenting cells and the route of infection have been suggested as possible predisposing factors.

It has long been problematic whether T cells are capable of recognizing polysaccharide antigens or sugars. Earlier studies on antibody responses to pneumococcal III polysaccharide indicated a role for T suppressor cells *in vivo* although there was no evidence for recognition of SSS-III by T cells¹⁵. Clearly antibody responses to hapten coupled-ficoll¹⁶ and the streptococcal A carbohydrate¹⁷ have been found to be T-cell dependent. Delayed type hypersensitivity to tuberculo-carbohydrates have been reported¹⁸. Recently, cell-mediated immunity to *Bacillus fragilis* has been shown to be induced by the capsular polysaccharide and, curiously, is mediated by a major histocompatibility complex unrestricted, Lyt2 T cell in the mouse¹⁹. These findings indicate that some carbohydrate moieties can be recognized by T cells, perhaps preferentially by T cells of the suppressor phenotype.

This work was supported by USPHS Grants A107118, A120111, A122682, and the World Bank/UNDP/WHO Special Programme for Research and Training in Tropical Diseases.

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Intrathymic presentation of circulating non-major histocompatibility complex antigens

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Intrathymic selection of T-cell specificity has been shown to be influenced by self-major histocompatibility complex (MHC) antigens encoded by radioresistant thymic stromal cells^{1,2}. The role of non-MHC antigens in intrathymic T-cell differentiation, in particular induction of antigen-specific tolerance, has been unclear³⁻⁵ and the access of non-MHC antigens to the thymus is controversial. Here we present evidence that circulating protein antigens enter the thymus and are presented by thymic stromal cells. At least three distinct types of stromal cells are thought to be associated with intrathymic lymphopoiesis⁶⁻¹²; after intravenous (i.v.) injection of antigen only I-A/E-positive medullary dendritic cells, but not I-A/E-negative macrophages or I-A/E-positive cortical epithelial cells co-purified with antigen-specific stimulation of cloned T-helper cells *in vitro*. Antigen presentation by thymic stromal cells was dependent on the dose of antigen injected and the time interval after injection.

Three types of direct cell-cell lymphostromal interactions have been identified as sites of intrathymic lymphopoiesis: these involve associations of thymocytes with (1) thymic macrophages (MØ), (2) thymic medullary dendritic cells (DC), and (3) cortical epithelial cells⁶⁻¹². Two types of multicellular lymphostromal complexes which represent the *in vitro* correlates of these interactions *in vivo* have been separately isolated and purified: (1) MØ and DC form rosettes with thymocytes (thymocyte rosettes, T-ROS) and (2) epithelial cells completely enclose the associated thymocytes (thymic nurse cells, TNC)¹⁰⁻¹². The T cells associated with all three types of stromal cells are specifically enriched in actively dividing cells¹². The recognition signals responsible for the binding and activation of the stromal cell-associated thymocytes have not been defined. From previous indirect evidence in chimaeras^{1,2}, it is surmised that self-MHC determinants, at least in part, specify these interactions. Here we analyse a possible role of blood-borne non-MHC antigens in these interactions.

Highly purified T-ROS are significantly enriched in antigen-presenting cells (APC) compared with unselected thymocytes. As few as 1×10^3 T-ROS elicit a stronger response than 1×10^6 unselected thymocytes, indicating a 100-1,000-fold enrichment of thymic APC in the rosette fraction (Fig. 1). The rosette-associated APC activity is unimpaired after removal of rosetting thymocytes by anti-Thy 1.2 antibodies plus complement, and >90% suppressed after treatment with anti-I-A antibody plus

Received 27 October 1983; accepted 3 January 1984.

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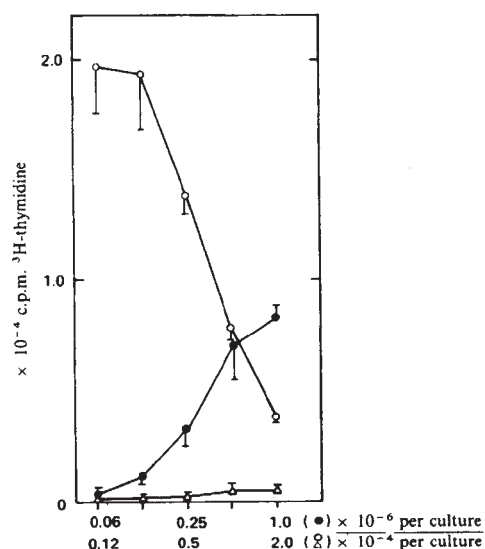


Fig. 1 Enrichment of thymic APC. Purified T-ROS (○) and unselected thymocytes (●) were irradiated (2,000 rad, Cs source) and co-cultured in graded numbers in triplicate cultures with GAT-specific I-A^b-restricted cloned T-helper cells (clone 14) (1×10^4 per culture), in round-bottomed 96-well plates. GAT (Miles Yeda; final concentration $200 \mu\text{g ml}^{-1}$ was added at the start of the culture, but not to control cultures containing T-ROS as stimulators (△). After 72 h proliferation was assayed by uptake of ^3H -thymidine in a 6-h pulse. Data represent the mean values of triplicate cultures $\pm$ s.d. Note that T-ROS and unselected thymocytes are titred at different concentrations.

Methods: Lymphostromal complexes were isolated and purified as described previously^{10,12} and used as stimulator cells in a proliferation assay of antigen-specific I-A-restricted T-helper cell clones. Antigen was either added at the beginning of the culture period *in vitro* or injected *in vivo* at various time intervals before isolation of the stimulator cells. Three soluble molecules of different molecular weights (MWs) were used as antigens: sperm whale myoglobin (Mgb), MW 17,000; the random co-polymer GAT, MW 60,000–100,000; KLH, MW 8×10^6 . MHC-restricted T-helper clones of the following specificities were used: myoglobin and I-A^b (clone 28.1), GAT and I-A^b (clone 14), KLH and I-A^{b,k} (clone NA 4.3) and KLH and I-A^b (clone 16.3)^{25–27}. In this experiment, 20 thymuses of C57BL/Ka mice (4–5-week-old females were used throughout all experiments) were pooled, minced and subjected to the following dissociation scheme: mild agitation for 10 min at 25°C in RPMI buffered with HEPES (Gibco), pH 7.3 (0.5 ml per thymus). The free cell suspension obtained after this step yielded the unselected thymocyte cell fraction. The remaining tissue fragments were then enzymatically dissociated as follows: twice for 10 min at 25°C and once for 10 min at 30°C with collagenase IV (0.5 mg ml^{-1} ; Millipore) and once for 10 min at 30°C in Dispase (0.3 mg ml^{-1} ; Boehringer). After each digestion step the cell suspensions were separated from the tissue fragments. The four cell fractions were pooled and thymocyte rosettes were purified by repetitive unit gravity sedimentation on one-step fetal calf serum gradients as described previously¹². Purification refers to a ratio of 1–3 free cells per T-ROS complex.

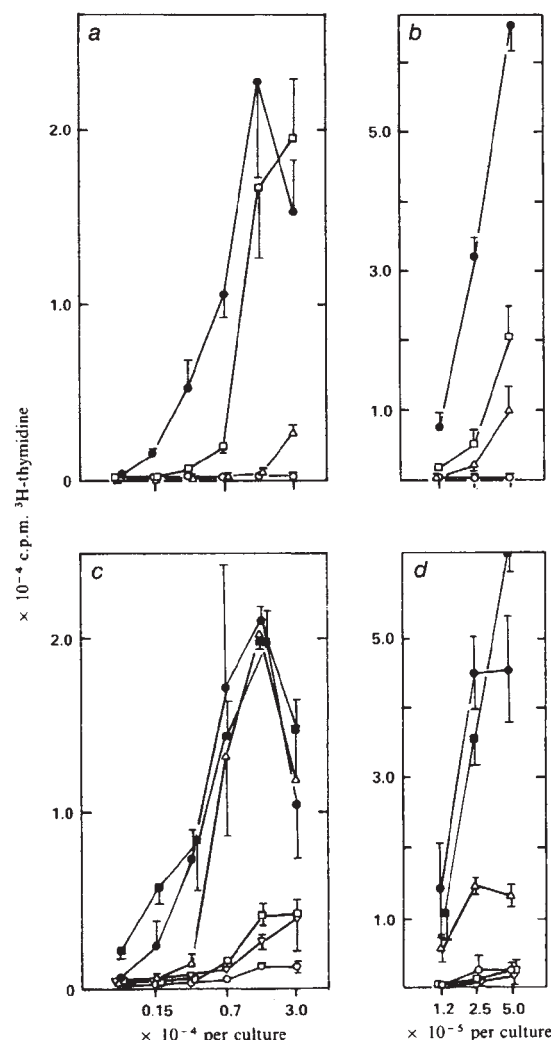


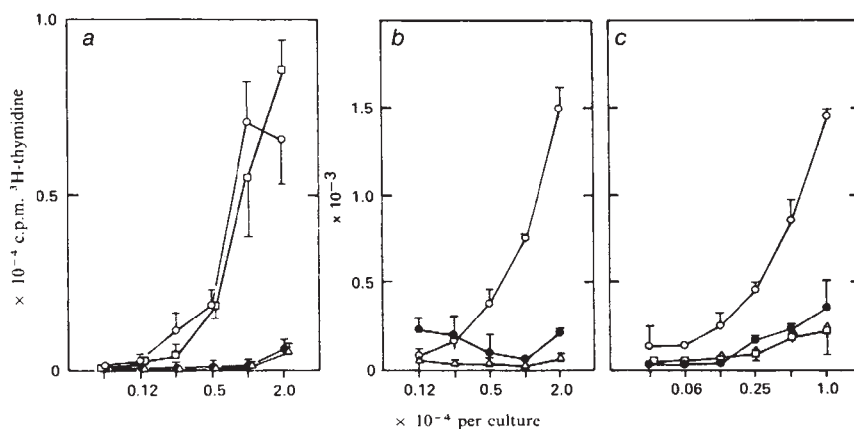
Fig. 2 *a, b*, Dose-response relationship of antigen traffic. Ten C57BL/Ka mice were injected either with 1.0 mg (●), 0.5 mg (□) or 0.25 mg (△) sperm whale myoglobin (Sigma) per g body weight or with phosphate-buffered saline (PBS, ○) and 2 h later the thymuses of each group were removed and pooled. T-ROS were purified (see Fig. 1 legend) and incubated for 1.5 h on glass coverslips. Stimulator cells to be tested for induction of proliferation were irradiated and co-cultured in graded numbers with 1×10^4 per culture of myoglobin-specific I-A^b-restricted cloned T cells (clone 28.1) and proliferation was assayed 72 h later. *a*, Non-adherent T-ROS (a mixture of DC-ROS, non-adherent Mφ and thymocytes). *b*, Splenocytes of the same groups. *c, d*, Time kinetics of antigen traffic. C57BL/Ka mice were injected with 0.5 mg per g body weight myoglobin *in vivo* and after 15 min (■), 2 h (●), 12 h (△), 24 h (□) and 48 h (▽) the thymuses of each group (10 mice per group) were pooled, and the T-ROS were isolated and purified. ○, Uninjected control. Stimulator cells used in the co-cultivation assay were: *c*, non-adherent T-ROS; *d*, splenocytes.

complement (data not shown). Thus, APC activity resides in the I-A-positive stromal cell fraction. To assess the physiological function of these thymic APC, we tested whether they are accessible to circulating antigens *in vivo* or are secluded by a blood-thymus barrier.

When myoglobin was injected *in vivo*, and 2 h later T-ROS were purified and used as stimulator cells in a proliferation assay *in vitro*, the relevant myoglobin-specific clone was stimulated in an antigen-dependent H-2-restricted fashion. The extent of T-cell proliferation was dependent on the dose of injected antigen with a minimal detectable response after injection of 0.25 mg antigen per g body weight and a plateau response above 1.0 mg myoglobin per g body weight (Fig. 2*a*). A similar dose-response relationship was found with unpurified splenic APC

from the same animals (Fig. 2*b*). When time kinetics of antigen presentation by thymic APC were measured with a standard dose of 0.5 mg myoglobin per g body weight, antigen reached the thymus as early as 15 min after injection (earliest time point tested), and a specific response was detectable for as long as 48 h after the antigen pulse (Fig. 2*c*). The rapid detection of antigen associated with thymic APC argues for a direct entry of free myoglobin and against an entry route via circulating antigen-laden APC¹³. The prolonged presence of antigen in the thymus for 48 h makes it unlikely that thymic APC, otherwise secluded from antigen, are secondarily exposed to high levels of antigen in contaminating blood during tissue disruption. Myoglobin is rapidly cleared from the circulation by the kidneys and only trace amounts should be present 12 h after injection,

Fig. 3 Cellular characterization of thymic APC. *a*, Twelve C57BL/Ka mice were injected with 0.5 mg myoglobin per g body weight and after 2 h the thymuses were removed, pooled and T-ROS were isolated and purified. Two-thirds of the purified T-ROS were incubated on glass coverslips in 24-well plates (Costar). After incubation for 1.5 h, the non-adherent cells were collected by gentle pipetting. The adherent cells were then removed by vigorous pipetting after incubation in 5 mM EDTA-PBS (Ca^{2+} , Mg^{2+} -free) for 5 min at 37 °C. Stimulator cells used in the co-cultivation assay were: intact purified T-ROS (○), non-adherent cells (□), adherent stromal cells (●), and adherent stromal cells after injection of myoglobin *in vivo* and additional antigen supply *in vitro* (20 µg myoglobin per culture) (△). *b*, *c*, (C3H × C57BL/Ka) F_1 mice were lethally irradiated (900 rad) and reconstituted with 10^7 C3H bone marrow cells (pretreated with anti-Thy 1.2 and complement); 10 weeks after reconstitution T-ROS and TNC were isolated and purified from the pooled thymuses. TNC were isolated by further dissociation of the thymic tissue remaining after isolating T-ROS as described previously¹². Ten P → F_1 chimaeras, seven untreated F_1 and nine C3H mice were injected with 0.5 mg myoglobin per g body weight 2 h before removal of the thymuses. Highly enriched T-ROS (*b*) and TNC (*c*) of all three experimental groups were used as stimulator cells in the co-cultivation assay. *b*, Response elicited by T-ROS derived from F_1 hybrid (H-2^{b,k}) (○), C3H (H-2^k) (●) and C3H → F_1 mice (△). *c*, Response elicited by TNC derived from F_1 hybrid (○), C3H (●) and C3H → F_1 (□) mice.



at which time the thymic APC are still as efficient in antigen-specific stimulation as at 15 min after antigen pulse (see Fig. 2c). L-glutamic acid⁶⁰-L-alanine³⁰-L-tyrosine¹⁰ (GAT) and keyhole limpet haemocyanin (KLH) were also shown to enter the thymus when 0.75–2.0 mg per g body weight of purified antigen was injected *i.v.* and 1–2 h later thymic rosettes were co-cultured with the appropriate T-helper clones (data not shown). Note that the threshold doses for detection of GAT and KLH in the thymus were 2–4 times higher than for detection of these antigens in the spleen. Thus, traffic of soluble blood-borne antigens to the thymus is time- and dose-dependent and is observed with antigens of a wide molecular weight range.

To delineate the cell type(s) responsible for antigen presentation, MØ, DC and epithelial cells were analysed separately. When the stromal cells of highly enriched T-ROS were separated into adherent (~90% macrophages) and non-adherent cells (~50–80% DC-like cells), antigen presentation *in vitro* co-purified with the non-adherent DC-rich cell fraction (Fig. 3a). Adherent stromal cells, even pulsed with additional antigen *in vitro*, did not stimulate the T cells (Fig. 3a). Thus, the failure of thymic MØ to present antigen is probably not due solely to inaccessibility to antigen *in vivo* and is compatible with their lack of class II MHC antigen expression¹². This finding is at variance, however, with earlier reports which used different enrichment protocols and ascribed thymic APC activity to an adherent cell population having macrophage characteristics^{14,15}.

Second, we separately tested I-A-positive cortical epithelial cells and I-A-positive DC-like cells. A complete separation of both cell types can be achieved in bone marrow-reconstituted radiation chimaeras in which epithelial cells of the host type remain and DC are replaced by bone marrow donor cells^{12,16}. We tested thymic APC activity in C3H (H-2^k) → (C3H × C57BL/Ka) F_1 (H-2^{k,b}), 900-rad, bone marrow chimaeras 10 weeks after reconstitution, with the myoglobin-specific I-A^b-restricted clone 28.1. As expected, APC in the rosette fraction did not present myoglobin to the I-A^b-restricted T-cell clone as both DC and MØ in the chimaeric mice were of H-2^k donor type (Fig. 3b). However, enriched F_1 hybrid host-derived epithelial cells, which strongly express I-A^b determinants serologically together with I-A^k and I-A^{b,k} hybrid determinants, did not induce myoglobin-specific proliferation above the control level of C3H-derived epithelial cells carrying the irrelevant H-2^k haplotype (Fig. 3c; the residual APC activity in the F_1 -derived TNC fraction is due to contaminating DC). Again, this failure of epithelial cells to present antigen cannot be attributed solely to sequestration of antigen. Epithelial cells derived from chimaeric mice of the same type also failed to present antigen when KLH was added *in vitro* and the

proliferation of the I-A^{b,k} hybrid determinant-KLH-specific T-cell clone was measured in two control experiments (data not shown). Thus, thymic APC seem to be strictly of bone marrow origin as reported previously¹⁶.

The demonstration of antigen within the thymus was critically dependent on the efficient purification of APC from pooled organs after enzymatic dissociation. It is unlikely that the APC activity described in these experiments is derived either (1) from perithymic lymph node cells, as care was taken to remove adjacent tissues, moreover DC-T-cell rosettes cannot be isolated in appreciable numbers even from primed peripheral lymph nodes (B.A.K., unpublished observation) or (2) from co-isolated vascular endothelial cells or pericytes, as neither of these cell types expresses class II MHC antigens of bone marrow origin constitutively¹⁷.

Thus, thymic dendritic cells are accessible to circulating antigens *in vivo* and efficient in the presentation of these antigens to T cells *in vitro*^{18,19}, and presumably also *in vivo*. In contrast, I-A-negative macrophages and I-A-positive cortical epithelial cells were intrinsically deficient in antigen presentation in our assay, indicating that I-A expression is necessary but not sufficient for antigen presentation. It is worth noting that thymic dendritic (interdigitating) cells are strictly confined to the medulla and corticomedullary junction^{8,9,20}, both of which have been shown to be permeable to protein antigens, whereas I-A-positive TNC reside in the cortex¹¹ which is apparently secluded from blood-borne antigens by a specialized vascular architecture²¹. These findings are compatible with a proposed role for thymic epithelial cells in selection of T cells via MHC determinants in the absence of nominal antigens^{1,2}.

Penetration of protein antigens into the thymus, particularly in close association with blood vessels in the medulla, has been reported previously^{21–24}. Our results provide evidence that blood-borne non-MHC antigens can reach thymic DC, which are highly efficient in presenting these antigens to T cells. The strict correlation of thymocyte-DC interactions with T-cell maturation during ontogeny strongly suggests a role of these stromal cells in T-cell lymphopoiesis.

We thank M. Travis for technical assistance, D. Logan for typing the manuscript, T. Infante and M. Shigeta for providing T-cell clones, and P. Fink and J. Trotter for helpful criticism on the manuscript. This work was supported by the E. Naumann fund and grant CA 03352 to H.S.K., grants A1 18705, 18635 and 00485 to C.G.F. and a postdoctoral fellowship of the Deutsche Forschungsgemeinschaft to B.A.K.

Received 1 September; accepted 21 December 1983.

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Growth regulation of a cellular tumour antigen, p53, in nontransformed cells

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Many transformed cells in culture have been found to express elevated levels of a cellular tumour antigen, termed p53¹⁻⁹. This protein has also been implicated in the regulation of cellular growth¹⁰⁻¹². For these reasons experiments were designed to examine the expression of p53 as quiescent cultures of nontransformed 3T3 fibroblasts were stimulated to reenter the cell cycle. Synchronous populations of cells were obtained by releasing a culture from density-dependent inhibition of growth with the addition of fresh serum¹³. Steady-state levels of p53 protein and mRNA were measured as a function of time after addition of serum to quiescent cultures and the rate of synthesis of p53 protein was analysed at a number of time points. The results, reported here, demonstrate an increase in the synthesis and steady-state levels of p53 protein and mRNA prior to DNA synthesis in late G₁, and suggest a role for p53 in the progression of cells from a growth-arrested state to an actively dividing state.

Confluent monolayers of 3T3 cells were stimulated to enter S phase by a serum deprivation/addition regime described in the legend to Fig. 1. Steady-state levels of p53 were analysed at various times after serum addition. p53 was immunoprecipitated from cellular extracts, electrophoresed in polyacrylamide gels and identified by the Western blotting technique¹⁴ (Fig. 1). By 6 h after serum addition there was a marked increase in the levels of p53 and just before entry into S phase the levels had increased by 10-20-fold. In this experiment 75% of the cells entered the S phase as detected by autoradiography of cell cultures after ³H-thymidine incorporation.

To examine whether the elevation in p53 levels after serum addition was due to increased synthesis of the protein, cultures were pulse-labelled with ³⁵S-methionine. p53 was immunoprecipitated from cellular extracts and electrophoresed in acrylamide gels. In the experiment in Fig. 2, the level of labelled p53 protein increased by fivefold within 11 h after the addition of fresh serum. It then appeared to decrease slightly as the cells progressed through DNA synthesis. These results suggest that the increase seen in steady-state levels of p53 protein is due to an increase in the rate of synthesis of p53 and not merely to accumulation of the protein. However, there appears to be a difference in the relative levels of increase of

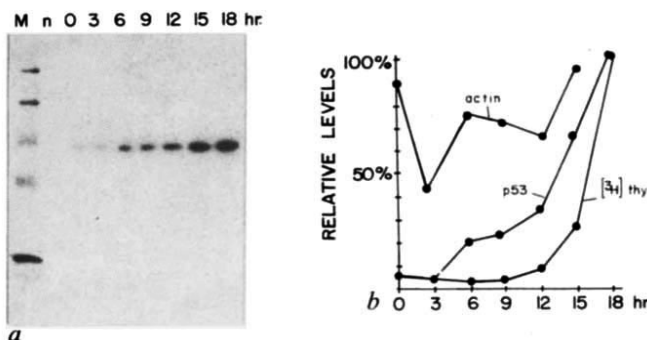


Fig. 1 Western blot analysis of p53 protein levels after serum stimulation of 3T3 cells. *a*, Autoradiogram of p53 Western blot showing times after serum stimulation: M indicates molecular weight size markers; n indicates immunoprecipitation with a control monoclonal antibody (2A6)²² 18 h after serum addition. *b*, Graphic representation of relative levels of p53 protein and actin protein (from densitometer scan of autoradiograms), and ³H-thymidine incorporation.

Methods: Synchronony was induced in Swiss 3T3 cells by replacing the media of confluent monolayers with median containing 0.5% fetal bovine serum for a period of 15-20 h. Cultures were then stimulated to enter DNA synthesis by the addition of 15% serum. Cellular extracts were prepared at various time point after serum addition. P53 protein was immunoprecipitated from the same amount of total cellular protein from each sample (2.3 mg) with an excess of anti-p53 monoclonal antibodies, PAb421^{3,21}. Immunoprecipitates were electrophoresed in SDS-polyacrylamide gels and processed for Western blot hybridization essentially as described by Burnette¹⁴. Proteins in the gel were electrophoretically transferred to a sheet of nitrocellulose. The immobilized antigens were visualized by incubation with PAb421 antibodies and the subsequent binding of ¹²⁵I-labelled *Staphylococcus aureus* protein A. Total cellular protein (0.1 mg) from each time point was analysed for actin protein by a separate Western blot hybridization. Equal amounts of protein were electrophoresed in SDS gels and transferred to nitrocellulose¹⁴. Actin protein was detected after reaction of the nitrocellulose with murine anti-actin antibodies (gift of D. Welsh), followed by goat anti-murine antibodies and ¹²⁵I-labelled *Staphylococcus aureus* protein A. The onset of DNA synthesis was measured by metabolic labelling of duplicate cell cultures with 5 μ Ci ml⁻¹ [³H]-thymidine for 20 min and analysing trichloroacetic acid (TCA) precipitable radioactivity.

p53 and the time of appearance of this increase compared with the results of the Western blotting experiment. This difference may reflect a lower sensitivity of the *in vivo* metabolic labelling technique or an accumulation of the protein due to increased stability.

To test for the possibility of an increased stability of p53 protein in late G₁, the half life of p53 was examined by pulse-chase experiments. The data presented in Fig. 3 show that either early or late in G₁ the half life of p53 is approximately 25 min. The half life was the same as late as 20 h after serum addition (data not shown). Stability of this protein, therefore, does not seem to contribute to the increase in p53 protein levels.

Since the increase in the steady-state levels of p53 appeared to be due to increased synthesis and not merely to accumulation of the protein, the levels of p53 mRNA were examined during 3T3 cell serum stimulation. The p53 mRNA species were identified by Northern blot hybridization¹⁵ using a nick-translated p53 specific cDNA clone as a probe¹⁶ (Fig. 4a). A single poly(A)⁺ mRNA species was detected migrating just ahead of an 18S rRNA marker. p53 mRNA levels were seen to increase by 6-7 h after serum addition and achieved a 10-20-fold increase just before the S phase. The transcripts of two other genes were also analysed over this time course (Fig. 4b). The levels of β -actin mRNA were low at the time of serum addition, but rapidly rose (by 3 h) and remained relatively constant. Histone H3 mRNA expression was tightly linked to DNA synthesis, as has been reported previously¹⁷.

To determine whether the increase in p53 protein and mRNA was independent of entry into S phase, a serum stimulation

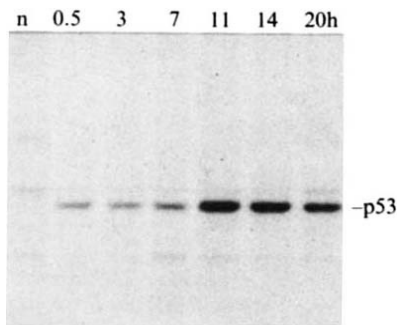


Fig. 2 p53 protein synthesis after serum stimulation of 3T3 cells. Autoradiogram of SDS polyacrylamide gel showing p53 immunoprecipitated from cells pulse-labelled 0.5, 3, 7, 11, 14 and 20 h after serum additions. n Indicates immunoprecipitation with control serum²².

Methods: After synchronization as described in the legend to Fig. 1, BALB/c 3T3 cell cultures were pulse-labelled with ³⁵S-methionine for 20 min at various times after serum addition. Cell extracts were prepared and the same amount of TCA precipitable radioactivity from each time point was reacted with anti-p53 monoclonal antibodies. The immunoprecipitates were analysed on SDS-polyacrylamide gels³.

experiment was performed in the presence of 1.5 mM hydroxyurea. This DNA synthesis inhibitor did not affect the pattern of increase in p53 protein and mRNA levels seen in the previous experiment.

The results of these studies demonstrate a regulated expression of p53 in nontransformed 3T3 cells. After experimentally inducing synchrony by serum-mediated stimulation of quiescent cells (G_0/G_1) the levels of p53 protein and mRNA are found to increase dramatically in late G_1 . The increase in p53 mRNA abundance is probably responsible for the elevation in p53 protein levels since the half life of p53 remains very brief (25 min) both early and late in G_1 . The time of elevated expression of p53 may therefore correlate with a time of functional activity of the protein. As with all synchronization protocols, the synchrony of the population deteriorates with time. For this reason it was difficult to examine precisely p53 expression after cells progressed into S phase and mitosis. However, the transition from G_0/G_1 into S phase did reveal a 10–20-fold increase in p53 expression in late G_1 . These results are in agreement with previous studies by Milner and co-workers who reported an increase in p53 synthesis after mitogenic stimulation of lymphocytes^{10,11}.

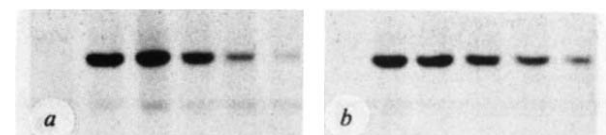
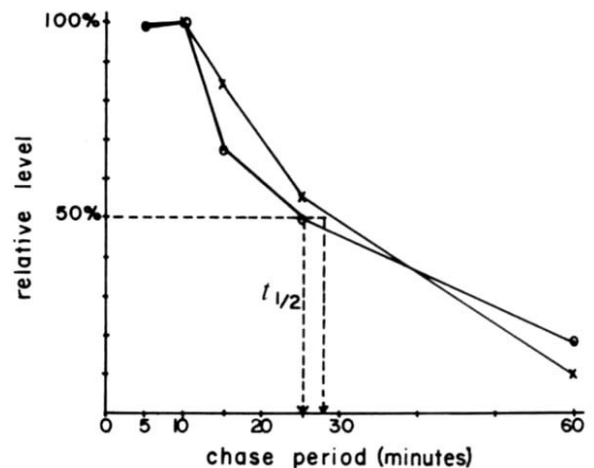


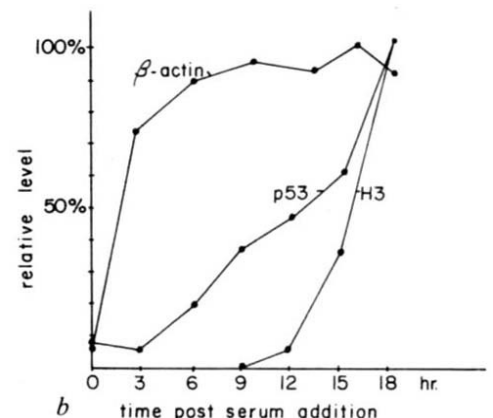
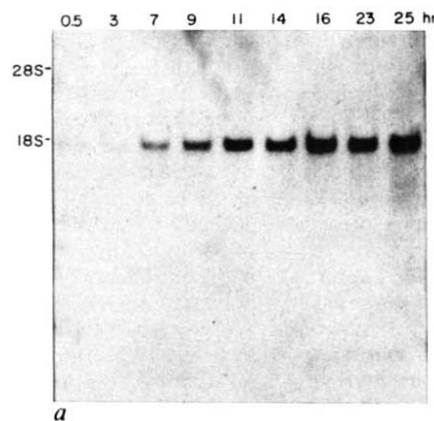
Fig. 3 The graph shows a pulse-chase analysis of p53 half life during serum stimulation of 3T3 cell. Graphic representation of the intensity of p53 protein signal: ○, cultures labelled at 2–3 h; ×, cultures labelled at 12–13 h. $t_{1/2}$ Indicates time of chase when half the initial labelled p53 protein is present. Also shown are autoradiograms of pulse-chase experiment. Left lane is immunoprecipitation with control serum. Sequential lanes are p53 specific immunoprecipitations at 5, 10, 15, 20 and 60 min of chase. a, 3 h post-serum; b, 13 h post-serum.

Methods: Synchrony was induced in Swiss 3T3 cells (legend to Fig. 1) and at either 2 or 12 h after serum addition, cultures were pulse-labelled with ³⁵S-methionine for 1 h. The cultures were then rinsed extensively in nonradioactive medium with an excess of methionine and incubated in this medium for the indicated time periods (chase). The same amount of TCA precipitable radioactivity was used from each sample for immunoprecipitation of p53 and analysis by SDS gels³.

Experiments suggesting a functional importance of the p53 molecule during serum induction of DNA synthesis have been reported by Mercer and co-workers¹². Anti-p53 antibodies microinjected into the nuclei of cells near the time of serum stimulation significantly reduced the percentage of cells entering DNA synthesis, but had no effect if microinjected 4 h or later

Fig. 4 Northern blot hybridization analysis of p53 mRNA levels after stimulation of quiescent cells. a, Autoradiogram of p53 mRNA. Northern blot showing time points after serum stimulation. The migration of rRNA size markers is indicated. b, Graphic representation of relative levels of RNA determined by densitometer tracing of the autoradiograms.

Methods: Cytoplasmic RNA was isolated from BALB/c 3T3 cells at various times after serum stimulation⁸. Poly(A)⁺ containing RNA was selected by chromatography over oligo(dT)-cellulose²³. The same amount (1 µg) of RNA from each time point indicated was denatured in 2.2 M formaldehyde, 50% formamide, 0.2 M MOPS, 50 mM sodium acetate, 1 mM EDTA, pH 7.0 at 70 °C for 5 min. The samples were electrophoresed in a 1% agarose-formaldehyde/MOPS buffered gel. The RNA species were transferred to nitrocellulose and hybridized with nick-translated plasmid DNA pp53-208¹⁶ essentially according to Thomas¹⁵ with the omission of dextran sulphate. In a separate experiment total cytoplasmic RNA was isolated from Swiss 3T3 cells after serum stimulation. 25 µg of RNA from each time point was analysed by Northern blot hybridization with plasmid DNAs containing cloned β -actin cDNA (pA1)¹⁷, cloned histone H3 cDNA (pST419-D) (gift of J. Stein and G. Stein) or cloned p53 cDNA¹⁶.



after serum addition. However, as shown here, the levels of p53 are very low 0–4 h after serum addition and only increase in late G₁. Perhaps p53 function is required continually throughout the G₁ period or at both the G₀/G₁ and G₁/S transition periods. It is also possible that the p53 antigen levels are in excess of the binding capacity of the microinjected antibody 4 h or more after serum addition, thereby overcoming the functional inhibition.

It is clear that p53 expression is regulated in nontransformed cells both at the level of protein turnover⁸, and, during the transition from a resting to a growing state, at the level of mRNA abundance. In contrast, transformed cells appear to have altered this normal regulation of p53 expression^{8,9,18}. The SV40 large T antigen has been shown to stimulate cellular DNA synthesis on microinjection into quiescent cell cultures¹⁹. Since T-antigen complexes with p53 protein *in vivo*^{1,2,20} it is possible that the induction of DNA synthesis is actually signalled by the increase in p53 stability and consequently p53 levels. Similarly, the higher steady-state levels of p53 found in nonviral transformants may signal continuous rounds of cell division and thereby prevent the entry into G₀. If this hypothesis is correct, the altered control of p53 expression would have a key role in the altered control of cell division in the transformed state.

We thank P. Sarnow, T. van Dyke and F. Chaudry for helpful discussions, and A. Teresky, R. Pashley, J. Demian and G. Urban for technical assistance. The research was supported by National Cancer Institute grant CA28127-03.

Received 15 September; accepted 16 December 1983.

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Mechanism of mutagenesis by O⁶-methylguanine

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O⁶-methylguanine (O⁶meG) lesions of double-stranded DNA have been associated with mutation^{1–3} and neoplastic transformation^{4,5}. These lesions can, in principle, be produced by at least three different mechanisms: direct alkylation of G·C base pairs in double-stranded DNA^{6,7}; alkylation of guanine residues in single-stranded regions of DNA associated with replication forks^{8,9}; and alkylation of the DNA precursor pool¹⁰ followed by incorporation of O⁶-methyl deoxyguanosine triphosphate

(O⁶-medGTP) during DNA replication. DNA biosynthesis subsequent to all three events will generate predominantly O⁶-meG·T base pairs as O⁶meG preferentially pairs with T^{11,12}. We show here that O⁶meG·T base pairs are mutagenic; that transalkylase repair^{1,13,14} has a direct role in the generation of mutations induced by alkylated pool nucleotides; and that the *Escherichia coli* mismatch repair system^{15,16} is capable of repairing mutagenic G·T intermediates.

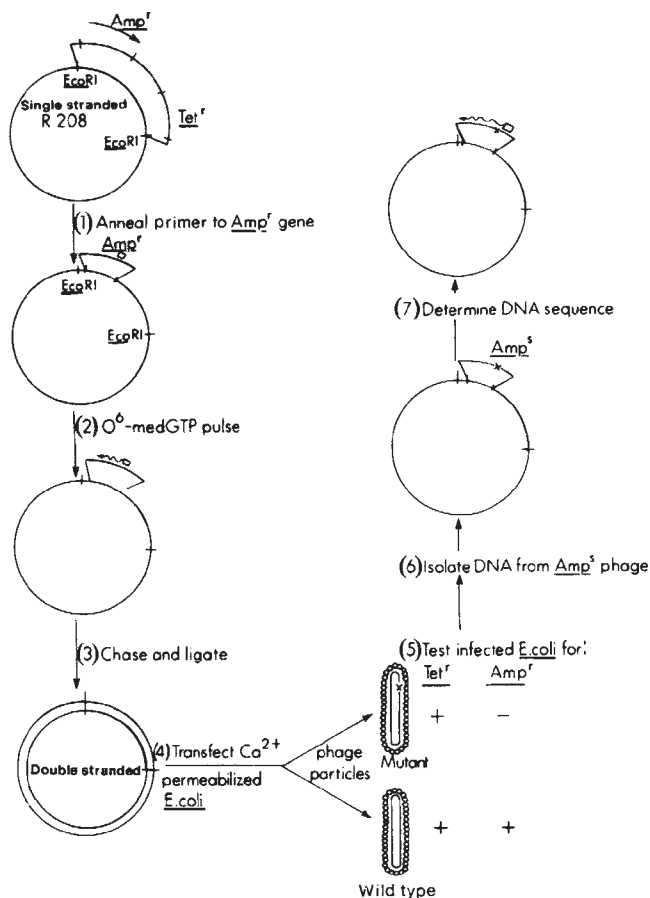
The mutagenicity of O⁶meG lesions was analysed using a forward mutation assay (Fig. 1), which makes use of the single-stranded bacteriophage f1/pBR322 chimera designated R208¹⁷. The pBR322 insert of R208 carries two selectable genes, *Amp^r* and *Tet^r*, which confer resistance to the antibiotics ampicillin and tetracycline, respectively, on the infected host bacteria. The single-stranded form of R208 allows the incorporation of modified DNA precursors at any desired region to be investigated by annealing to the appropriate primer and also facilitates nucleotide sequencing by the dideoxy technique¹⁸.

To analyse the mutagenicity of O⁶meG lesions in DNA, O⁶medGTP (synthesized and purified as described elsewhere¹²) was incorporated into the *Amp^r* gene of R208 during a pulse of DNA replication ('minus' strand synthesis, see Fig. 1) to give predominantly O⁶meG·T, and a small amount of O⁶meG·C, base pairs¹². This method allowed determination of the mutagenicity of O⁶meG lesions in DNA without the complication of the additional types of lesions formed by direct alkylation. The pulse of DNA replication in the presence of O⁶medGTP was followed by a 'chase' in the presence of an excess of unmodified dNTPs to convert the primed single-stranded DNA to double-stranded molecules, which were used to transfect various *E. coli* strains (discussed below). Infective progeny phage (a product of rolling-circle 'plus' strand synthesis) were collected and screened for their ability to produce an active ampicillinase (β -lactamase) by the iodine decolorization method¹⁹ modified for plaque assays. Transfer of plaques to tetracycline and ampicillin plates was used to quantitate by an independent method the percentage of mutants (*Tet^r*, *Amp^s*) and to isolate mutant clones which had not been subjected to iodine treatment for DNA sequencing.

Results obtained with this mutation assay indicate that O⁶meG lesions do not markedly increase the background mutation rate in repair competent *E. coli* strains (data not shown). This result is consistent with the repair of O⁶meG·T and O⁶meG·C lesions by transalkylase activity to give either a wild-type G·C base pair or, more frequently, the mismatched base pair G·T. Mismatched base pairs are repaired by the *E. coli* mismatch repair system^{16,20}, directed by undermethylation of GATC sites of the *in vitro* synthesized nascent strand, which contains the error²⁰.

In the absence of mismatch repair G·T mismatches would remain in the transfected DNA, so we investigated the effect of transfecting *E. coli mutH* strains^{21,22}, which lack mismatch repair, with O⁶meG-containing R208 DNA. The results (Table 1) demonstrate a significant level of O⁶medGTP-induced mutagenesis in *mutH* strains. The observed variation in the mutagenesis rate with the concentration of unmodified dNTPs in the replication pulse (Table 1) is consistent with the DNA synthesis arrest that can follow O⁶medGTP incorporation¹². O⁶medGTP arrests DNA synthesis when incorporated *in vitro* at particular DNA sequences. The presence of dATP allows synthesis past such potential arrest sites by incorporating in place of O⁶medGTP either directly or after O⁶meG removal at arrest sites by the 3'–5' exonuclease activity of DNA polymerase (unpublished results). An equal concentration of dATP and O⁶medGTP was found to be the most mutagenic—higher concentrations of dATP decrease O⁶medGTP mutagenesis by competitively reducing O⁶medGTP incorporation. The determination of the DNA sequence changes in eight randomly selected mutant R208 progeny showed that all mutations were A·T to G·C transitions (see Fig. 2 for a representative autoradiogram) as predicted by the following pathway for the formation of G·T

Fig. 1 Methodology of the mutagenicity assay. Single-stranded R208 DNA¹⁷ was used as a template for *in vitro* DNA synthesis. A 12 nucleotide primer strand complementary to positions 3,467–3,478 (pCAATAAACCCAGC) in the ampicillinase gene of pBR322²⁸ was treated with polynucleotide kinase and then annealed to the template by incubation with a 20-fold excess of primer at 25 °C for 20 min in buffer containing 100 mM NaCl, 10 mM Mg²⁺, 1.0 mM β -mercaptoethanol, and 20 mM Tris-HCl pH 7.4 (step 1). The annealed primer/template was then used for *in vitro* polymerization reactions (steps 2 and 3). The pulse reactions contained primer/template DNA (0.15 μ g ml⁻¹), 13 mM Mg²⁺, 1.0 mM dithiothreitol (DTT), 80 mM NaCl, 10 μ M O⁶medGTP (where indicated in Table 1), equimolar concentrations of all four normal dNTPs as indicated (Table 1), 13 mM Tris-HCl pH 7.4 and the Klenow fragment of DNA polymerase I (0.6 U μ l⁻¹). Separate pulse reactions were continued for 2 min and 10 min at 24 °C, and then an equal volume of 'chase' mix was added containing 1.1 mM each normal dNTP, 6.6 mM Mg²⁺, 1.0 mM DTT, 50 mM NaCl, T₄ polynucleotide ligase (0.2 U μ l⁻¹), Klenow enzyme (0.08 U μ l⁻¹), and 6.6 mM Tris-HCl pH 7.4 (step 3), and incubation was continued for a further 10 h at 24 °C. For transfection (step 4) the 2 min and 10 min polymerase pulse reactions were pooled, diluted 15-fold with 50 mM Tris-HCl pH 7.6, and desalted through Sephadex G-25-80. *E. coli* strains were prepared for transfection by the CaCl₂ method as described previously²⁹. 75 μ l of filtrate containing about 1.5 μ g of DNA were added to 100 ml of cells on ice for 15 min. After 1 min at 45 °C, TYE media (1.8 ml) were added and cultures were incubated at 37 °C. Infected centres were plated at 18 min and progeny phage plated after 3 h. *E. coli* strain K38³⁰ (provided by B. Webster) was used as an indicator strain. Phage stocks were prepared by centrifugation at 10,000 r.p.m. for 5 min. The decanted supernatant was stored at 4 °C. In step 5 progeny phage were tested for the mutant phenotype (Tet^rAmp^s). For rapid screening a modification of the method of Boyko and Ganschow¹⁹ was used. Plaque assay plates containing 1.5% starch in the top agar were flooded with 2.0 ml of iodine assay solution as described¹⁹. Plaques formed by wild-type phage decolorize the stain while mutant phage plaques remain dark and are easily identifiable. For isolation of mutants for sequencing, plaques were toothpicked onto tetracycline plates (10 μ g ml⁻¹) and, after growth, were replica-plated onto ampicillin plates (500 μ g ml⁻¹). Mutant progeny (Tet^rAmp^s) were amplified and the DNA isolated and sequenced by standard techniques (steps 6 and 7).



intermediates:



The presence of only A·T to G·C transitions is consistent with transalkylase-mediated conversion of O⁶meG·T lesions to mutagenic G·T intermediates. To investigate the role of the 'adaptive response' in the establishment of O⁶medGTP-induced mutagenesis we transfected strains deficient in transalkylase activity with O⁶meG-containing DNA. These *E. coli* *ada* strains, PJ3, PJ5 and PJ6^{23,24}, are sensitive to both the mutagenicity and toxicity of methylating agents. They have been shown to lack the adaptive response in the order PJ6 < PJ3 < PJ5 < parental strain by mutability with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG)²³. Transfection of *E. coli* *ada* strains with O⁶meG-containing R208 DNA showed increasing mutagenesis with decreasing capacity for the adaptive response (Table 1).

Mutagenesis in transalkylase-deficient strains was postulated to be the result of the inherent ambiguity of the unrepaired O⁶meG lesion, which has been predicted to give equivalent levels of A·T to G·C and G·C to A·T transition mutations¹². However, all 15 nucleotide sequence changes identified in mutants isolated from the *ada* strain least capable of the adaptive response^{24,25}, PJ6, were A·T to G·C transitions (data not shown), which may be due to constitutively expressed transalkylase activity. A reduced cellular capacity for induction of transalkylase repair may allow sufficient time for *in vivo* methylation of the nascent, undermethylated strand by dam methylase²⁶ prior to transalkylase repair. In the absence of mismatch correction²⁰ transalkylase repair of O⁶meG·T sites will leave uncor-

rectable G·T intermediates, and so A·T to G·C transitions will predominate. In addition, preferential repair (either transalkylase or direct mismatch correction) of O⁶meG·C lesions could also play a part in the elimination or reduction below detectable levels of G·C to A·T transition mutations.

To confirm the effect of mismatch repair attenuation in a transalkylase deficient strain, R208 DNA was methylated *in vitro* using dam methylase prior to transfection of the *ada* strains. Fully methylated GATC sites effectively eliminate mismatch repair²⁰. As observed in *mutH* hosts, lack of mismatch repair should increase O⁶meG-induced mutagenesis. Dam methylation of R208 DNA increased the mutation rate in the *ada* strains, their parental strains and the *mutH* parental strain three- to six-fold (Table 1), but had no effect on the *mutH* strain as it already lacked mismatch repair. When five sequence changes were located in dam methylated-R208 mutants isolated from PJ6, again all changes were A·T to G·C transitions (data not shown).

Our findings demonstrate that O⁶meG lesions in DNA can give rise to mutations *in vivo* via G·T intermediates. This result suggests that introduction of this lesion into DNA by the incorporation of O⁶medGTP from the alkylated precursor pool can initiate a molecular pathway for A·T to G·C transition mutations. As G·C to A·T transitions have been shown to occur at least 10–20 times more frequently than A·T to G·C transitions at a given site after cellular exposure to high concentrations of MNNG²⁷, direct alkylation of single- or double-stranded DNA may be the major pathway for this mutagenesis. Nevertheless, at low levels of mutagen, insufficient to saturate transalkylase, O⁶meG·C base pairs should be demethylated to non-mutagenic G·C base pairs. O⁶meG·T base pairs resulting from alkylation

Table 1 Mutagenicity of *O*⁶medGTP

Genotype of host strain*	Concentration of dNTPs (μM)	% Mutants†		Ratio of % mutants ± <i>O</i> ⁶ medGTP
		+10 μM <i>O</i> ⁶ medGTP	− <i>O</i> ⁶ medGTP	
<i>mutH</i>	0.01	0.3 ± 0.2	0.2 ± 0.2	1
<i>mutH</i>	0.1	0.4 ± 0.3	0.2 ± 0.1	2
<i>mutH</i>	0.5	1.1 ± 0.4	0.2 ± 0.2	6
<i>mutH</i>	1.0	1.4 ± 0.2	0.3 ± 0.3	5
<i>mutH</i>	10	1.9 ± 0.8	0.1 ± 0.1	19
<i>mutH</i>	50	0.3	0.4	1
<i>mutH</i>	200	0.3	0.1	3
		Methylated	Unmethylated	Ratio methylated:unmethylated
<i>ada</i> parent	10	1.6	0.27	6
<i>ada-5</i>	10	1.6	0.45	ND
<i>ada-3</i>	10	1.9	0.58	3
<i>ada-6</i>	10	2.8	1.1	3
<i>mutH</i> parent	10	0.84	0.20	4
<i>mutH</i>	10	2.3	2.1	1

Indicated methylation was carried out *in vitro* with purified dam methylase (a gift of P. Modrich) as described elsewhere²⁶ except cold *S*-adenosyl methionine was used. Dam methylase was omitted from control reactions. ND, not determined; mutation rate was assumed to be at the background level observed for the *ada* parent.

* Strains used: *mutH*, RH213²¹ (provided by B. Bachmann) and KMBL3773 and its parent KMBL 3752²² (provided by P. Modrich); *ada*, PJ3, PJ5, PJ6 and their parent AB1157²³ (provided by B. Sedgewick).

† Range, where given, indicates an average of three experiments (two with KMBL 3773 and one with RH213).

‡ Average of five experiments with KMBL 3752 (*mutH* parent) or KMBL 3773 (*mutH*).

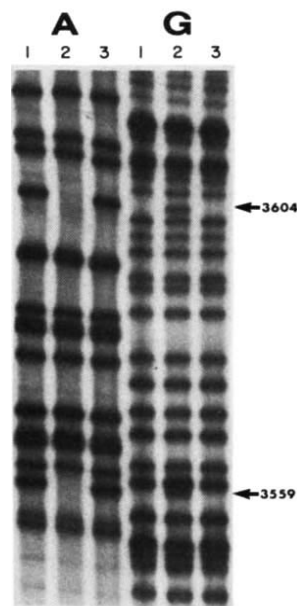


Fig. 2 Audioradiogram of representative DNA sequencing run. Shown are the A and G lanes for wild type (lane 1), mutant 10-1 (lane 2), and mutant 6H-19 (lane 3). Arrows indicate positions of A to G transition mutations in mutant 10-1. Numbers indicate the pBR322 coordinate²⁸ of the mutations. Mutant 6H-19 was wild type in the region shown. C and T lanes (not shown) were also wild type for both mutants.

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Formation of the 3' end of histone mRNA by post-transcriptional processing

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The specific 3' termini of a number of eukaryotic mRNAs have been shown to be generated by the post-transcriptional processing of primary transcripts or pre-mRNAs¹⁻⁴. The sequence AAUAAA, present in the 3' region of nearly all eukaryotic mRNAs, appears to be involved in the cleavage and subsequent polyadenylation of the primary transcript⁵. An exception to this general rule is the case of the histone mRNAs, which lack the AAUAAA sequence and are not normally polyadenylated. Histone mRNAs do, however, contain a highly conserved 23 base pair sequence at their 3' termini⁶, which is required for correct 3' end formation⁷. The similarity between this conserved sequence, which can be drawn as a hairpin loop, and bacterial

of single-strand DNA should yield G·C to A·T transitions whether or not *O*⁶meG·T base pairs are converted to G·T base pairs by transalkylase. *O*⁶meG·T base pairs resulting from *O*⁶medGTP incorporation will yield A·T to G·C transitions upon demethylation by transalkylase as demonstrated above. The relative contribution of these two mutagenic pathways at low mutagen levels is unknown.

We thank Clyde Hutchison III and Paul Modrich for helpful suggestions and Sandra J. Johnson for technical assistance. This work was supported by USPHS grants GM24798 and CA28632 (M.D.T.), and NCI postdoctoral fellowships 5 T32 CA09156 (J.S.E.) and 5 F32 CA07108 (D.T.). M.D.T. is a Leukemia Society of America Scholar.

Received 15 August; accepted 13 December 1983.

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transcription terminators⁸ has led several investigators to suggest that the specific 3' end of histone mRNA is formed by termination of transcription^{9,10}. So far, however, experimental results have not been presented which make it possible to distinguish between a post-transcriptional processing or a transcription termination mechanism for the formation of histone mRNA 3' termini. We have investigated this issue by synthesizing *in vitro* unprocessed histone pre-mRNAs that extend past the normal 3' terminus. These *in vitro* synthesized pre-mRNAs were injected into frog oocyte nuclei to study their fate. The results demonstrate that correct 3' ends of chicken histone H2B mRNAs can be formed by RNA processing of longer synthetic pre-mRNAs.

Previous studies have shown that authentic sea urchin H2A and H2B histone mRNA are produced when the sea urchin histone DNA repeat is injected into oocyte nuclei¹¹. The present experiments have been performed with a chicken histone H2B gene. This gene was inserted into a vector containing a bacteriophage SP6 promoter, generating the hybrid plasmid pSP62-H2B shown in Fig. 1. The chicken H2B DNA portion of this plasmid contains about 100 base pairs (bp) of 5' flanking region, including the TATA box and other conserved promoter sequences⁶, and ~1,400 bp of 3' flanking sequence. This plasmid serves a dual purpose in our experiments. First, nascent histone mRNA transcripts can be synthesized *in vivo* from the histone promoter when pSP62-H2B DNA is injected into oocyte nuclei. Second, pSP62-H2B can function as a transcription template for the *in vitro* synthesis of histone H2B pre-mRNAs using the SP6 promoter and purified SP6 RNA polymerase^{12,13}.

To determine whether the chicken histone gene can direct the synthesis of authentic histone mRNA in frog oocytes, the plasmid pSP62-H2B was injected into oocyte nuclei and total RNA was extracted and analysed by S₁ nuclease mapping 18 h later (Fig. 2). From the sequence of the chicken histone H2B gene¹⁴ we predicted an S₁-protected fragment of 116 bases if the 5' end of the histone transcript is correctly initiated in injected oocytes. Correct formation of the 3' end of the histone transcript should result in an S₁-protected fragment of 87 bases. Both of these predicted S₁ fragments are indeed observed as major bands when pSP62-H2B DNA is injected into oocytes. These bands are not observed when uninjected oocyte RNA is analysed (Fig. 2). The pSP62-H2B transcripts do not appear to be polyadenylated after 18 h as judged by oligo-dT cellulose chromatography (data not shown).

Bands at the position of fully protected probe (217 or 143 bases) indicate the presence of readthrough transcripts, probably resulting from transcription initiation within the vector sequences of the plasmid rather than at the chicken histone promoter. These readthrough transcripts have not been mapped, but have been observed in many other oocyte injection experiments (reviewed in ref. 15).

The 5' and 3' termini of authentic chicken histone H2B mRNA have not been directly mapped, but the highly conserved nature of histone genes allows the 5' and 3' ends of the natural histone mRNAs to be predicted with confidence. The 5' ends of histone mRNAs map within a conserved region that has the consensus sequence CCATT^{16,17}. Moreover, S₁ mapping experiments¹⁶ and sequencing of histone cDNAs^{18,19} have shown that histone mRNAs end with the sequence ACC(C)A. This terminus is located at the 3' end of the 23 base histone mRNA 3' consensus sequence. The transcripts produced in injected oocytes (Fig. 2) have 5' and 3' termini that are entirely consistent with these predictions. We conclude that oocytes initiate transcription at the correct position on chicken histone genes and, more pertinently, the resulting histone mRNAs contain correct 3' termini. These conclusions are in complete agreement with R. Sturm's unpublished studies on chicken histone H2B expression in injected oocytes and with the extensive studies of Birnstiel and his colleagues on sea urchin histone genes^{11,16}.

While it has been demonstrated that the conserved sequence block adjacent to the 3' end of histone mRNA is involved in 3' terminus formation⁷, no experimental evidence has yet been

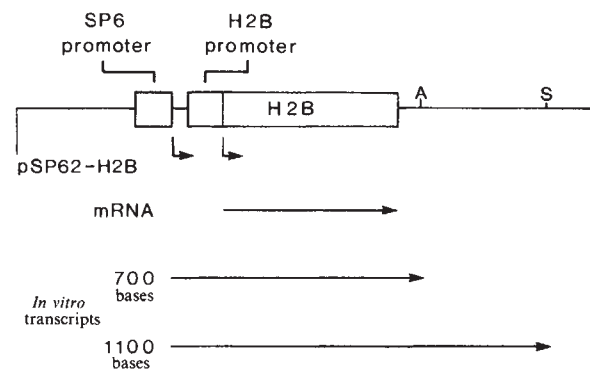


Fig. 1 Transcription map of the chicken H2B gene-containing plasmid pSP62-H2B. RNA transcription *in vitro* directed by the SP6 promoter initiates ~140 bp upstream of the authentic H2B mRNA initiation site. *In vitro* transcripts of sizes 700 and 1,100 bases are synthesized using pSP62-H2B templates cut with *Aha*III (A) and *Sac*II (S) respectively. Plasmid pSP62-H2B was constructed by inserting a promoter of the *Salmonella typhimurium* bacteriophage SP6 into the vector pSP62 (an *Amp*^r gene-containing derivative of a previously described SP6 vector¹³), and inserting downstream from this promoter a 1.9 kb fragment of chicken histone DNA isolated from clone λ CHO5¹⁴.

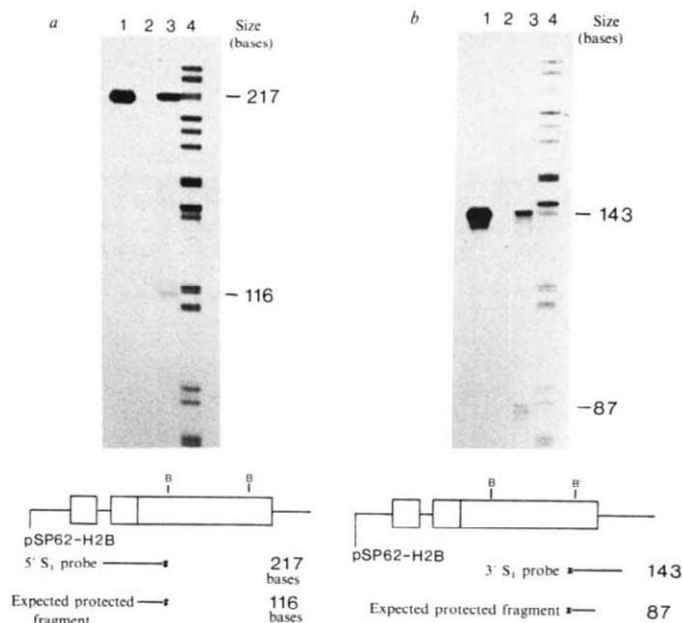


Fig. 2 Transcription of chicken histone DNA in microinjected oocytes. The figure shows the results of S₁ nuclease mapping of the 5' (a) and 3' (b) ends of RNA transcribed from pSP62-H2B in *Xenopus* oocytes, using the probes shown below the gels. Lanes 1, undigested ³²P-labelled probe; lanes 2, labelled probe mixed with uninjected oocyte RNA and digested with S₁ nuclease; lanes 3, S₁ nuclease digested probe protected with RNA extracted from oocytes injected with pSP62-H2B; lanes 4, ³²P-labelled markers. In the diagram below the gels the asterisks mark the ³²P-labelled ends of the probes, and Bs indicate *Bst*EII sites on pSP62-H2B. **Methods:** Approximately 5 ng of pSP62-H2B DNA was injected into *Xenopus* oocyte nuclei, which were then incubated for 18 h and RNA was extracted from them as described previously²¹. The 5' and 3' probes were labelled at *Bst*EII sites within the H2B gene.

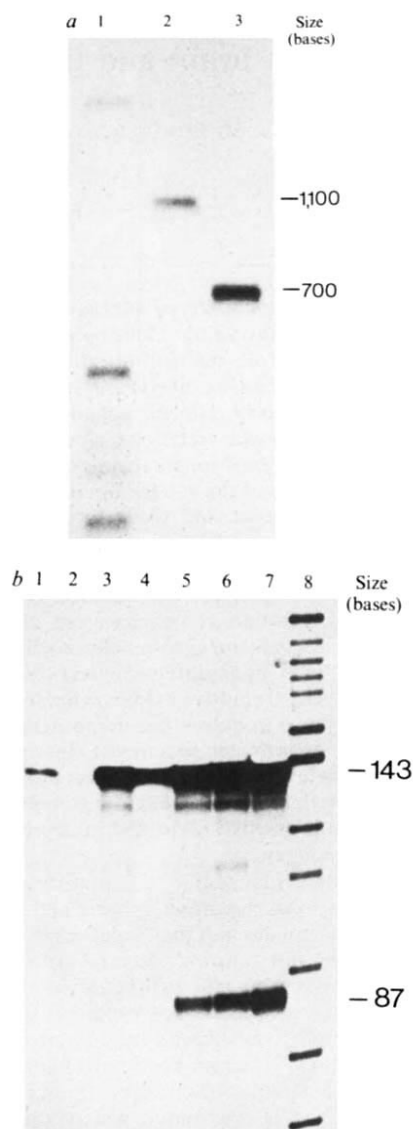


Fig. 3 *a*, *In vitro* synthesis of chicken H2B pre-mRNA. Lane 1, ³²P-labelled markers; lane 2, transcription from pSP62-H2B cut with *Sac*II; lane 3, transcription from pSP62-H2B cut with *Aha*III. *b*, Processing of *in vitro* transcribed H2B pre-mRNAs in injected *Xenopus* oocyte nuclei. Lane 1, ³²P-labelled *S*₁ probe (3' probe shown in Fig. 2); lane 2, *S*₁ digested probe mixed with uninjected oocyte RNA; lanes 3-7, *S*₁ digested probe that had previously been mixed with: lane 3, uninjected 1,100 base transcripts; lane 4, uninjected 700 base transcripts; lane 5, RNA from oocytes injected with the 1,100 base transcripts; lane 6, RNA from oocytes injected with 700 base transcripts; lane 7, RNA from oocytes injected with pSP62-H2B; lane 8, ³²P-labelled markers.

Methods: For *a*, the transcription reactions were performed at 37 °C for 1 h with 1 µg of linear DNA and 4 U of purified SP6 RNA polymerase in 50 µl of the following solution: 40 mM Tris HCl, pH 7.5, 6 mM MgCl₂, 10 mM dithiothreitol (DTT), 2 mM spermidine, 50 U RNAsin, 400 µmol of each ribonucleoside triphosphate, 10 µCi [α -³²P]GTP. Vanadyl ribonucleoside was then added to 10 mM, and the DNA template digested with DNase (20 µg ml⁻¹) at 37 °C for 10 min. After phenol:chloroform extraction 1-3 µg of the ³²P-labelled RNA was purified by Sephadex G100 chromatography. For all injection experiments a 5' cap was added to the RNA with guanylyl transferase (5.6 U) at 37 °C for 45 min in 50 mM Tris-HCl, pH 7.9, 1.25 mM MgCl₂, 2.5 mM DTT, 50 µM *S*-adenosyl methionine, 500 M GTP, 30 U RNAsin. The *in vitro* transcripts were run on a 1.8% agarose gel containing formaldehyde. For *b*, 2 ng of *in vitro* transcripts were injected into *Xenopus* oocyte nuclei, and after a 4 h incubation RNA was extracted and *S*₁ nuclease mapped as described in the legend to Fig. 2. The products of the *S*₁ digestions were run on a 10% denaturing acrylamide gel.

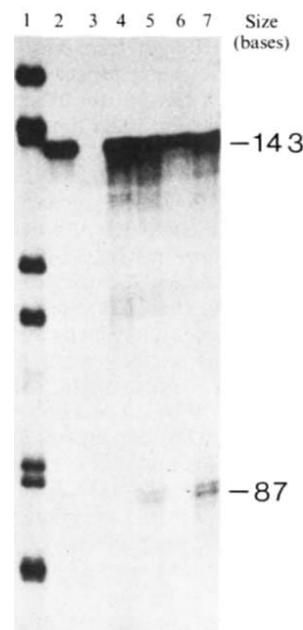


Fig. 4 Association of the 3' processing activity with the oocyte nuclei. Lane 1, ³²P-labelled markers; lane 2, ³²P-labelled probe (same as in Fig. 3b); lanes 3-7, *S*₁ nuclease digested probe mixed with: lane 3, uninjected oocyte RNA; lane 4, uninjected 1,100 base RNA; lane 5, RNA from nucleus-injected oocytes; lane 6, RNA from cytoplasm-injected oocytes; lane 7, RNA from pSP62-H2B injected oocytes.

Methods: 2 ng of *in vitro* transcribed H2B pre-mRNA were injected into either the nucleus or cytoplasm of *Xenopus* oocytes, and after a 4 h incubation the RNA was extracted and *S*₁ nuclease mapped as described in Fig. 2 legend. The *S*₁ digestion products were run on a 6% denaturing acrylamide gel.

obtained that shows whether the involvement is at the level of the DNA, as a signal for transcription termination, or at the level of the RNA transcript, as a recognition sequence for post-transcriptional processing. If the 3' end is normally formed by RNA processing, the substrate for that reaction should be a histone pre-mRNA containing a 3' end that extends beyond the 3' terminus of the mRNA. In order to distinguish between these two alternatives we synthesized *in vitro* RNA molecules extending well beyond the 3' end of mature H2B mRNA and injected these into oocytes to test for 3' processing.

Two different histone pre-mRNAs were synthesized using an SP6 transcription system^{12,13}. One of the DNA transcription templates was prepared by cutting pSP62-H2B at the unique *Sac*II site which is located ~460 bp downstream from the end of the histone H2B gene. The second template was prepared by cutting pSP62-H2B at the *Aha*III site which is located only 76 bp downstream from the H2B gene (Fig. 1). *In vitro* transcription of these two linear DNA templates with purified SP6 RNA polymerase results in the synthesis of discrete run-off transcripts the 5' ends of which correspond to transcription initiation at the SP6 promoter. The *Sac*II and *Aha*III cut templates direct the synthesis of SP6 transcripts which are 1,100 bases and 700 bases long, respectively (Fig. 3a). For convenience we refer to these *in vitro* synthesized RNAs as histone pre-mRNAs, noting that they contain ~140 extra bases 5' to the normal initiation site and 76 or 460 extra bases at the 3' end. In separate experiments, both *in vitro* synthesized histone pre-mRNAs were injected into oocyte nuclei and the RNAs were subsequently analysed by *S*₁ nuclease mapping. Figure 3b shows that the 87 base *S*₁ protected fragment, indicative of correct 3' end formation, is present when both the 1,100 and 700 base pre-mRNA transcripts are injected into oocyte nuclei. These data show that correct 3' ends of histone mRNA may be formed by post-transcriptional processing and that 3' end formation is therefore not obligatorily coupled to transcription or transcription termination.

The 700 base pre-mRNA appears to have been processed more efficiently than the longer transcript though in neither case are all the injected molecules processed. Subsequent time-course studies have shown that all the observed 3' processing occurs within 4 h after injection (data not shown). We find that both the pre-mRNA and the correctly 3'-cleaved RNA are quite stable in injected oocytes, though we have not carefully measured their half lives. Possible explanations for the incomplete processing of the injected RNA include the fact that a relatively large amount of pre-mRNA (2 ng) is injected in these experiments and this may exceed the oocyte's normal processing capabilities. Alternatively, the oocyte's 3' processing activity may be confined to the nucleus, in which case once pre-mRNAs leave the nucleus they may not be further processed.

To directly test whether 3' end formation occurs in the nucleus or the cytoplasm, the 1,100 base pre-mRNA was injected into either the nucleus or the cytoplasm and analysed as above. The results presented in Fig. 4 show that correct 3' end formation is observed only when the pre-mRNA is injected into the nucleus. The pre-mRNA injected into the cytoplasm is stable, but not processed. Thus, as previously shown for splicing activity^{13,20}, the 3' processing activity is associated with the nucleus.

In this report we have demonstrated that the oocyte can form correct 3' termini of histone mRNA when a longer, *in vitro* synthesized precursor RNA is injected into the oocyte nucleus. This shows that histone mRNA 3' ends may be formed by a mechanism other than termination of transcription. The processing of the precursor RNA to give authentic 3' termini was only observed when the RNA was injected into the nucleus, and while this does not prove that the processing actually occurs within the nucleus, it does show that a nuclear component is absolutely required. These results agree with the studies of Birnstiel and his colleagues who have recently shown that a small nuclear ribonucleoprotein (snRNP) isolated from sea urchin embryos is required to generate the correct 3' ends of histone H3 transcripts^{10,22}. Our results suggest that this snRNP is not a 'termination' factor^{10,22}, but rather acts at the level of RNA processing.

It remains possible that the 3' end of histone mRNA is also formed by termination of transcription, but the presence of an activity in the oocyte nucleus that is able to process longer histone transcripts to give accurate 3' ends is sufficient to account for the synthesis of histone mRNAs. We suggest that histone mRNAs are normally formed from pre-mRNAs which extend beyond the end point of the mRNA and which are cleaved to generate mature 3' termini.

We thank J. R. E. Wells and R. Sturm for the chicken histone H2B gene and unpublished restriction mapping data, and M. Green, T. Maniatis, G. Struhl and J. R. E. Wells for comments on the manuscript. This work was supported by grants from the Dreyfus Foundation, the Chicago Community Trust/Searle Scholars Program, and the NIH. P.K. is the recipient of an Australian CSIRO Postdoctoral Research Award.

Received 25 October; accepted 13 December 1983.

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Identity of angiotensinogen precursors of rat brain and liver

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Angiotensin modifies a number of functions of the central nervous system (CNS) including blood pressure regulation, thirst, and the secretion of the antidiuretic and adrenocorticotrophic hormones. Whereas peripheral administration of angiotensin produces these effects, accumulating evidence implicates a brain angiotensin system which operates independently of the circulating renin-angiotensin system (reviewed in refs 1-3). All components of the renin-angiotensin system have been identified in the brain, but there is considerable controversy over whether these components are synthesized in the brain or derived from plasma and the extent to which these components interact to generate angiotensin in the brain *in vivo*⁴. Whereas many different enzymes are able to cleave angiotensinogen (renin substrate) to release angiotensin I (AI) or angiotensin II (AII)^{3,5-11}, angiotensinogen is the only known precursor of AI and AII. Definitive evidence for an independent brain angiotensin system requires the demonstration that the brain synthesizes an angiotensin precursor. Here we report the identification of angiotensinogen mRNA in rat brain by cell-free translation and show that the brain angiotensinogen precursors are identical to those previously identified for liver¹², the source of plasma angiotensinogen.

There is convincing evidence that angiotensinogen is present in the CNS¹³⁻²⁵. However, the origin of brain and cerebrospinal fluid (CSF) angiotensinogens and their relationship with plasma angiotensinogen are not known. Several studies of CNS angiotensinogen have provided indirect evidence for local synthesis^{16,17,19,23} but they have not excluded the possibility that it may be derived from plasma by an actively regulated process²⁵. We sought evidence for synthesis of angiotensinogen by brain using the technique of cell-free translation of brain RNA. Angiotensinogen is distributed widely throughout the CNS and has been measured in all areas of the brain^{15-19,25}. Several studies have suggested that nephrectomy and glucocorticoid administration, two well-characterized stimuli of liver angiotensinogen synthesis, may also increase brain and CSF levels of angiotensinogen^{16,17,23}. Thus we prepared RNA from the whole brains of both normal rats and rats which had been nephrectomized and given dexamethasone 24 h prior to sacrifice (Nx/Dex rats).

We have previously shown by cell-free translation of liver RNA that rat liver synthesizes two angiotensinogen precursors, a major precursor molecular weight (MW) 52.5 K and a minor precursor MW 55.7K¹². Fig. 1 shows that identical precursor forms of angiotensinogen to those previously characterized for rat liver were immunoprecipitated from cell-free translations of rat brain RNA. Moreover, the relative abundance of the two precursors was similar for lysates primed with RNA from brain and liver. In three separate experiments, there was a small increase in the relative abundance of angiotensinogen precursors immunoprecipitated from cell-free translations of brain RNA from Nx/Dex rats compared with normal rats (Fig. 1, lanes 2 and 6) which was less than twofold (compare Fig. 1, lane 2 with lane 8). For the experiment shown in Fig. 1, translations were performed with 360 µg ml⁻¹ brain RNA. Similar results were obtained in experiments performed with 180 µg ml⁻¹ RNA, a concentration which produced less than maximal stimulation of translation. In contrast to the result shown for brain RNA, there was a large increase in translatable levels of angiotensinogen mRNA in livers of Nx/Dex rats compared with normal rats (Fig. 1, inset).

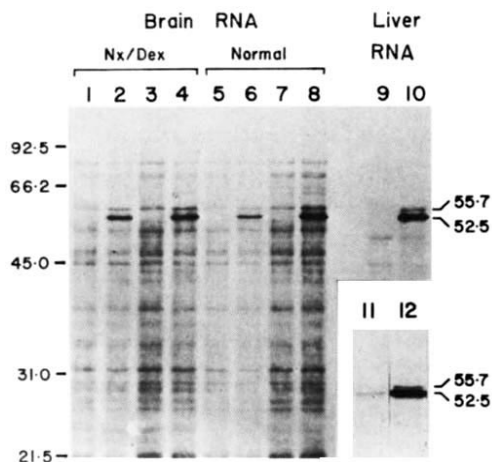


Fig. 1 Immunoprecipitation of angiotensinogen precursors from reticulocyte lysates primed with brain RNA prepared from nephrectomized, dexamethasone-treated (Nx/Dex) rats (lanes 1–4), brain RNA from normal rats (lanes 5–8) and liver RNA (lanes 9–10). 35 S-methionine-labelled immunoprecipitates were resolved by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and examined by autoradiography; exposure time 4 days. Immunoprecipitation was performed with either nonimmune rabbit serum (lanes 1, 3, 5, 7, 9) or angiotensinogen antibody ORNI (lanes 2, 4, 6, 8, 10). Lanes 1, 2, 5, 6, immunoprecipitation of 0.8×10^6 trichloroacetic acid (TCA)-insoluble c.p.m.; lanes 3, 4, 7, 8, immunoprecipitation of 1.6×10^6 TCA-insoluble c.p.m.; lanes 9, 10, immunoprecipitation of 1.8×10^5 TCA-insoluble c.p.m. The numbers on the right side of the autoradiograph indicate the molecular weight ($\times 10^{-3}$) of the angiotensinogen precursors based on their migration relative to the molecular weight standards (Bio-Rad) shown on the left side of the autoradiograph. Inset: Immunoprecipitation with antibody ORNI of reticulocyte lysates primed with RNA prepared from liver from a normal rat (lane 11) and a Nx/Dex rat (lane 12). Each lysate contained approximately $400 \mu\text{g ml}^{-1}$ RNA and showed similar translation efficiency. Both lanes show the immunoprecipitation of 4.5×10^5 TCA-insoluble c.p.m.; exposure time 12 days.

Methods: All tissues were obtained from male Wistar rats (body weight $\sim 300\text{g}$) fed a normal diet. RNA was prepared from the pooled brains (including anterior pituitary and cervical spinal cord) of four normal rats and four rats which had been nephrectomized and given dexamethasone sodium phosphate (Merck, Sharpe & Dohme, 7 mg kg^{-1}) by subcutaneous injection 24 h prior to sacrifice. Liver RNA (lanes 9, 10) was prepared from a rat which had been nephrectomized and given dexamethasone sodium phosphate (7 mg per kg), 17- α -ethynyl-estradiol (3 mg per kg), 3,3',5-triiodo-L-thyronine ($40 \mu\text{g per kg}$) and 0.15 M NaCl (100 ml per kg) by subcutaneous injection 24 h prior to killing. This procedure produces maximal stimulation of liver angiotensinogen production. RNA was prepared by the following modification of the method of Chirgwin *et al.*³⁴. After the second ethanol precipitation from 7.5 M guanidine hydrochloride, the RNA precipitate was washed once with 70% ethanol in water at -20°C , then digested with proteinase K (0.2 mg ml^{-1} in 10 mM Tris HCl, $\text{pH } 7.6$, 0.5% SDS) at 37°C for 60 min. The RNA was then extracted with phenol, precipitated thrice with 3 M sodium acetate, $\text{pH } 6$, and finally from 0.3 M sodium acetate, $\text{pH } 5.2$ with 2.2 volumes of ethanol at -20°C as described previously¹². The recovery of RNA from brains of normal rats ($363 \mu\text{g g}^{-1}$, wet weight) and Nx/Dex rats ($375 \mu\text{g g}^{-1}$, wet weight) was similar and both RNA preparations showed identical translation efficiency with half maximal stimulation of translation at $120 \mu\text{g ml}^{-1}$ and maximal stimulation at $360 \mu\text{g ml}^{-1}$ RNA. Recovery of liver RNA was 5 mg g^{-1} , wet weight. Maximal translation efficiency with $480 \mu\text{g ml}^{-1}$ liver RNA was approximately twice that achieved with brain RNA. The methods used for cell-free translation using nuclease-treated rabbit reticulocyte lysate, immunoprecipitation, SDS-PAGE on 10% gels and autoradiography have been described previously¹². Antibodies to pure rat plasma angiotensinogen²⁷ were raised in rabbits and showed a high specificity for rat plasma angiotensinogen but equal affinity for both angiotensinogen and des-(AI)-angiotensinogen³⁵.

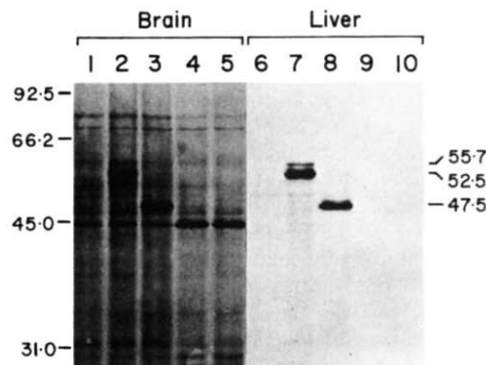


Fig. 2 Cleavage of angiotensinogen precursors by renin and effect of preabsorption of antibody with pure angiotensinogen. 35 S-methionine-labelled immunoprecipitates of cell-free translations of brain RNA from Nx/Dex rats (lanes 1–5) and liver RNA (lanes 6–10) were resolved by SDS-PAGE and visualized by autoradiography (see Fig. 1 legend). Lanes 1, 6, immunoprecipitation with nonimmune rabbit serum; lanes 2–5, 7–10, immunoprecipitation with angiotensinogen antibody ORNI; lanes 3, 5, 8, 10, lysate incubated with pure mouse submaxillary gland renin (640×10^{-3} Goldblatt Units ml^{-1}) for 30 min at 30°C before immunoprecipitation; lanes 4, 5, 9, 10, antibody preabsorbed with $20 \mu\text{g}$ pure rat plasma angiotensinogen²⁷ prior to immunoprecipitation. Lanes 1–5, immunoprecipitation of 4.5×10^5 TCA-insoluble c.p.m., exposure time 12 days; lanes 6–10, immunoprecipitation of 4×10^5 TCA-insoluble c.p.m., exposure time 2 days. Pure mouse submaxillary gland renin was prepared as described by Cohen *et al.*^{36,37}.

As shown in Fig. 2, both brain angiotensinogen precursors were cleaved by renin resulting in a single immunoreactive protein (des-(AI)-angiotensinogen) identical to that shown for liver, of MW 47.5K . Ohkubo *et al.*²⁶, from the nucleotide sequence of rat liver angiotensinogen mRNA, have concluded that the major 52.5K precursor does not contain a pro-sequence. Thus, given that renin cleaves rat angiotensinogen at a single site between amino acids 10 and 11 to release the decapeptide AI²⁷, we have proposed that the 55.7K precursor differs from the 52.5K precursor in that it contains a pro-sequence¹². Neither of the angiotensinogen precursors nor des-(AI)-angiotensinogen were immunoprecipitated by antibody preabsorbed with pure rat plasma angiotensinogen (Fig. 2).

The present study provides the first definitive evidence for extrahepatic synthesis of angiotensinogen and demonstrates that the angiotensinogen precursors synthesized in liver and brain are identical by several criteria including immunoreactivity, renin cleavage products and electrophoretic mobility. Given that angiotensinogen is the only known natural substrate for renin, the cleavage of both angiotensinogen precursors by renin represents a highly specific method for their identification. One consequence of the lack of a pro-sequence in the major angiotensinogen precursor²⁶ is that mature angiotensinogen is an obligatory product of cleavage of the signal peptide in those tissues, including brain, which synthesize this precursor. The similar molecular weight and electrophoretic mobility of CSF and plasma angiotensinogen²⁴ suggest that post-translational processing of the angiotensinogen precursors is similar for liver and brain. However, variation in glycosylation mechanisms could account for the differences in isoelectric points and affinity for concanavalin A previously reported^{19,20,24}.

The less than twofold increase in translatable brain angiotensinogen mRNA levels in response to nephrectomy and dexamethasone treatment is in contrast to the several-fold increase observed for liver (Fig. 1 and ref. 12) and suggests that, although brain and liver angiotensinogen mRNAs appear to be products of the same gene(s), the regulation of their transcription is tissue specific. The efficiency of cell-free translation of a particular mRNA is subject to many variables which

relate to both the translation system and the RNA preparation, and which preclude other than qualitative estimates of mRNA abundance. Further studies using a cDNA probe to measure angiotensinogen mRNA levels accurately in different brain regions are necessary to help define the role of this intrinsic brain angiotensinogen system.

The synthesis by liver and brain of two angiotensinogen precursors, differing presumably in the presence or absence of a pro-sequence, raises the possibility that these two precursors may be subject to different cellular processing pathways. Several studies suggest the intraneuronal generation of angiotensin^{2,28-32} and we speculated that the 55.7K precursor may contribute to intracellular generation of angiotensin and thus be relatively more abundant in brain. However, we were unable to demonstrate any major difference in the relative abundance of the two precursors for liver and brain. This finding does not exclude the possibility of intracellular generation of angiotensin. Ohkubo *et al.*²⁶ have previously noted that preangiotensinogen exhibits a molecular organization similar to that of the arginine vasopressin/neurophysin II precursor³³. By immunohistochemistry neuronal immunoreactive angiotensin has been localized to areas which also contain vasopressin^{2,28,29}. Thus peptidergic neurones containing angiotensin may possess the mechanism for intracellular cleavage of angiotensinogen to generate AI or AII which may then be released to perform a neuromodulator or neurotransmitter role in the CNS. Another possibility to be considered is that angiotensinogen synthesized in the CNS may serve a function unrelated to the renin-angiotensin system.

This work was supported by INSERM (France) grant CRL 824022 and Association Claude Bernard. We thank Simon Foote for the gift of pure mouse submaxillary gland renin, and Nicole Braure for typing the manuscript. D.J.C. is recipient of a C.J. Martin Fellowship from the National Health and Medical Research Council of Australia and a Bushell Travelling Fellowship of the Royal Australasian College of Physicians.

Received 19 September; accepted 13 December 1983.

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Clathrin light chains and secretory vesicle binding proteins are distinct

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Recently, several groups¹⁻⁴ have initiated studies on cytosolic proteins that bind to isolated secretory vesicle membranes in the presence of Ca^{2+} in order to identify proteins that may regulate exocytosis. Two major chromaffin granule binding proteins, of molecular weights 32,000 (32K) and 34,000 (34K), were reported to have the same mobility on one-dimensional SDS gels as clathrin-associated light chains from the adrenal medulla, and the 34K granule binding protein the same one-dimensional peptide map as the 34K clathrin light chain⁵. These observations support the hypothesis that Ca^{2+} -dependent recruitment of soluble light chains to the vesicle membrane may nucleate the assembly of a clathrin coat and initiate endocytosis. Here we report that two-dimensional peptide maps of the clathrin light chains and of all chromaffin granule membrane binding proteins in the 30K range are distinct, and therefore fail to support this hypothesis. It has also been suggested that some or all of the vesicle binding proteins require calmodulin for their interaction with the membrane⁵. However, we find that antagonism of calmodulin by trifluoperazine does not prevent the association of the other cytosolic proteins with the chromaffin granule membrane.

Secretory vesicle binding proteins were isolated by the technique of Ca^{2+} -dependent affinity chromatography of adrenal medullary cytosol on a column of chromaffin granule membranes that had been coupled to CNBr-activated Sepharose 4B (refs 1, 2). By applying cytosol to this column in the presence of 2 mM Ca^{2+} and then eluting the column with buffers with reduced Ca^{2+} concentrations, down to 0.1 μM , we isolated a class of cytosolic proteins that bind to the granule membranes in a Ca^{2+} -dependent manner. We refer to these proteins as chromobindins¹, in analogy with the established terms chromogranins⁶, the proteins in the granule core, and chromomembrins⁷, the proteins in the granule membrane, although the secretory vesicle binding proteins are not unique to chromaffin tissue (ref. 2 and Fig. 2). The chromobindins include calmodulin, synexin and protein kinase C². We have characterized approximately 20 additional chromobindins according to the concentrations of Ca^{2+} at which they elute from the affinity column, the Mg-ATP dependence of some of them to promote binding, and their affinity for membrane proteins or lipids². In addition, we have determined the apparent molecular weights and isoelectric points of the chromobindins separated on two-dimensional gels. As we reported previously², two chromobindins, referred to here as CB5 and CB7, are closest in positions on these gels to adrenal medullary clathrin light chains. The light chains, however, are slightly more acidic. One possible explanation for this difference is that the light chains are slightly modified forms of the granule binding proteins. We felt that two-dimensional peptide mapping might resolve this issue. Because there are six granule binding proteins in the 30K mass range², we felt it necessary to map all six proteins because any one or several could have contributed to the peptide maps previously published which were derived from protein bands on one-dimensional gels⁵.

The chromobindins and medullary clathrin light chains were excised from Coomassie-stained, polyacrylamide gels, iodinated, digested with trypsin and the tryptides separated on TLC plates by electrophoresis in one dimension and ascending chromatography in the second dimension⁸. Autoradiographs of the peptide maps of granule binding proteins CB5 and CB7 are shown in Fig. 1 and compared with maps of clathrin light chains. The patterns are clearly distinct. On longer exposure of the

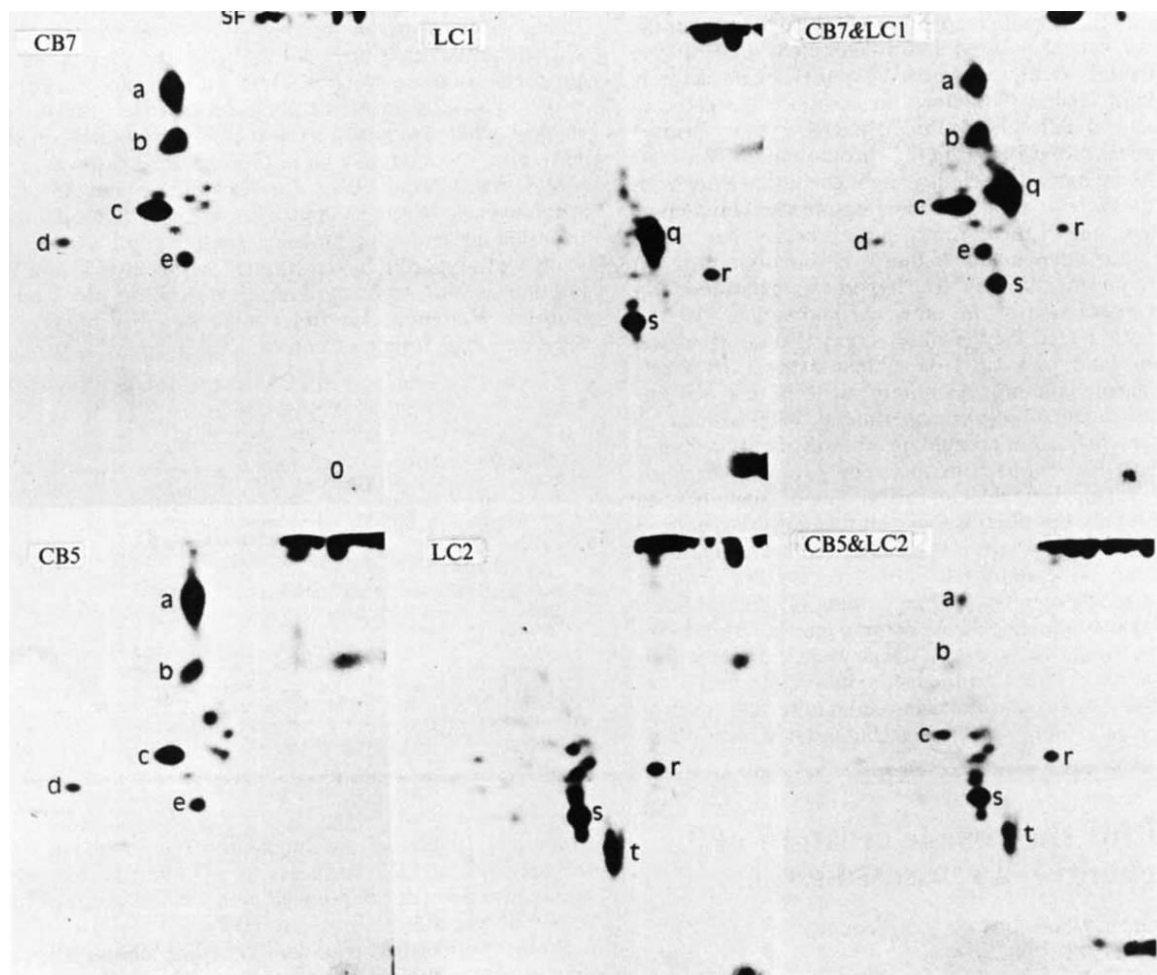
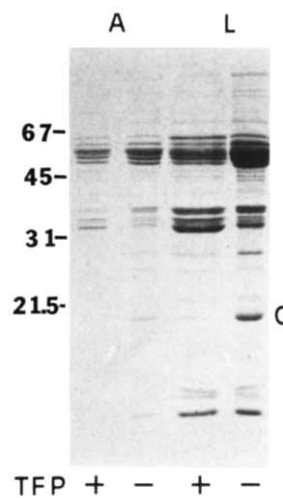


Fig. 1 Peptide maps of clathrin light chains (LC1, high molecular weight light chain; LC2, low molecular weight light chain) and membrane binding proteins CB5 and CB7. Each sample was spotted in the lower right hand corner ('O' in CB7), separated by electrophoresis to the left, followed by ascending chromatography to the solvent front ('SF' in CB7). 'CB7 & LC1' and 'CB5 & LC2' are maps of mixtures of the proteins of the same molecular weights. a, b, c, d, e: major peptides of the granule binding proteins; q, r, s, t: major peptides of the clathrin light chains.

Methods: Proteins CB5 and CB7 were excised from two-dimensional gels of chromobindin proteins isolated by affinity chromatography². Clathrin light chains were isolated as follows. Coated vesicles were prepared from 100 g of bovine adrenal medullary tissue following the procedure of Keen *et al.*⁹. Clathrin was extracted from the final vesicle pellet by incubating overnight at 4 °C with 0.5 M Tris, 2 M urea in Keen's buffer A (0.2 M 2-[N-morpholino]ethane sulphonic acid, 1 mM EGTA, 0.5 mM MgCl₂) adjusted to pH 7.5. The stripped membranes were sedimented at 100,000g for 60 min and the clathrin-containing supernatant was chromatographed on Sephadex G-25 to exchange the buffer for 50 mM ammonium acetate, pH 7.6. The extract was then incubated at 100 °C for 10 min¹³ and denatured protein sedimented at 100,000g for 60 min. The supernatant was lyophilized and analysed by one-dimensional electrophoresis in SDS¹⁴. The light chains appeared as two prominent bands in the 30K molecular weight range and were excised and mapped. Neither the 100 °C incubation nor the method of electrophoretic separation affected the maps, as similar patterns were obtained if the light chains were excised from one- or two-dimensional gels of the extract before heating. The electrophoretically purified proteins were iodinated, trypsinized and mapped according to Elder *et al.*⁸.

autoradiographs, additional peptide differences became apparent beyond those illustrated in Fig. 1. Maps were also prepared from the four other chromobindins of masses 32K to 39K; these were also distinct from those of the light chains. It is interesting to note the great similarity in the maps of proteins CB5 and CB7, while all of the other chromobindins that we have mapped were quite different from one another, in spite of their common property of Ca²⁺-dependent membrane association. The adrenal light chains appeared to have two major peptides in common, marked 'r' and 's' in Fig. 1. Brain clathrin light chains, which are of higher molecular weight than adrenal light chains (36K and 38K compared with 32K and 34K), and bovine muscle tropomyosin which has a similar subunit composition⁹, were also mapped and were found to be distinct from all granule binding proteins. However, the maps of brain light chains showed considerable homology with the adrenal light chains: peptides q, r, s and t (Fig. 1) were also present in the maps of the brain light chains. Three or four major additional peptides appeared in the brain light chain maps that were not seen in the maps of adrenal light chains.

Fig. 2 SDS gels of secretory vesicle membrane binding proteins isolated in the presence or absence of 100 µM trifluoperazine (TFP). Bovine adrenal medullary (A) or liver (L) cytosol was applied in the presence of 2 mM free Ca²⁺ to a column of chromaffin granule membranes coupled to Sepharose 4B². Ca²⁺-dependent membrane binding proteins were eluted with 2 mM EGTA. C marks the position of the calmodulin band; the numbers refer to the molecular weights (×10⁻³) of protein calibration standards. Electrophoresis was conducted according to Laemmli¹⁴ and the gels were stained with Coomassie blue. The minor changes seen at 10–12K and 38–39K in A, ±TFP, were not consistently observed (for example, compare with L, ±TFP).



In addition to the peptide mapping studies, we found that the chromobindins were denatured and precipitated by heating to 100 °C for 10 min, a treatment we used to purify the particularly heat-stable light chains. Therefore, in addition to significant primary structural differences, the light chains have distinct physical chemical properties from the chromobindins. We conclude that adrenal medullary clathrin light chains have not been obtained in the *in vitro* affinity chromatography experiments.

Calmodulin is one of the chromobindin proteins that can be isolated by Ca^{2+} -dependent affinity chromatography on chromaffin granule membranes². The hypothesis that calmodulin regulates the interaction of the other chromobindins with the membrane is supported by the observation that some of the chromobindins bind in a Ca^{2+} -dependent manner to a calmodulin-Sepharose column³. As a more stringent test, we conducted affinity chromatography experiments with adrenal or liver cytosol on chromaffin granule membrane affinity columns in the presence of 100 μM trifluoperazine to antagonize calmodulin action. This drug blocked the binding of calmodulin to the affinity columns, but did not significantly alter the spectrum of other chromobindins obtained (Fig. 2). Therefore, calmodulin does not appear to be required for the recruitment of these other proteins to the membrane. The binding of some of these proteins to a calmodulin-Sepharose column may have been due to their ability to interact in a Ca^{2+} -dependent manner with a hydrophobic surface, either on the calmodulin molecule, or on membrane lipid. The presence of calmodulin in the chromobindin class of proteins may reflect direct interaction with mem-

brane proteins, or an indirect interaction through the other chromobindins that does not influence the membrane binding properties of these proteins. Thus, with the exception of synexin which may initiate intermembrane contact and fusion¹⁰⁻¹², it is unclear what the roles of the membrane binding proteins we have characterized may be in the secretory pathway.

We thank James Keen for providing a sample of bovine tropomyosin, Virginia Simnad for help in the trifluoperazine experiments and Lisa Dowling for technical assistance. This study was supported by the NSF (PCM-82-06453), the Virginia Affiliate of the American Heart Association, the Council for Tobacco Research and the University of Virginia Diabetes Research and Training Center.

Received 13 October; accepted 5 December 1983.

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A model for the cosmic creation of nuclear energy—a reassessment

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Nature **296**, 540-543 (1982)

THIS letter included an unfortunate mistake which affects the loss of exergy in helium synthesis and hence the total exergy per nucleon. Naively one would expect the exergy per nucleon to be b_4 (the binding energy per nucleon in ^{56}Fe) apart from the loss due to helium formation. With a fraction γ_0 of neutrons immediately before the helium synthesis, this loss could be expected to be $2\gamma_0 b_3$ where b_3 is the ^4He binding energy per nucleon. Thus for a cooling universe ($T \rightarrow 0$), the exergy per nucleon $E(T)$ would approach

$$E(0) = b_4 - 2\gamma_0 b_3 = 6.36 \text{ MeV} \quad (11^*)$$

instead of the result (11) given in our paper. The simple estimate (11*) turns out to be the right one.

Consider a mixture of four ideal non-degenerate gases (consisting of ^1H , ^1n , ^4He and ^{56}Fe corresponding to $i = 1, 2, 3, 4$) in a volume V . Let n_i be the number of nuclei of the i th kind, so that in all there are $N = \sum_i n_i A_i$ nucleons. Since radiation dominates, equilibrium temperature and equilibrium pressure prevail, and the exergy per nucleon is purely 'chemical'.

$$E = \frac{1}{N} \sum_{i=1}^4 n_i (\mu_i - \mu_{i0}) = \frac{1}{N} \sum_{i=1}^4 n_i kT \ln \frac{n_i/V}{n_i^{(0)}/V}$$

Here μ_i is the chemical potential of nuclide i and μ_{i0} is its equilibrium value corresponding to an equilibrium density $n_i^{(0)}/V$. With

$$\begin{cases} P_i = \frac{n_i A_i}{N} \\ P_i^{(0)} = \frac{n_i^{(0)} A_i}{N} \end{cases}$$

the exergy may then be written

$$E = \sum_{i=1}^4 P_i \frac{kT}{A_i} \ln \frac{P_i}{P_i^{(0)}} \quad (2^*)$$

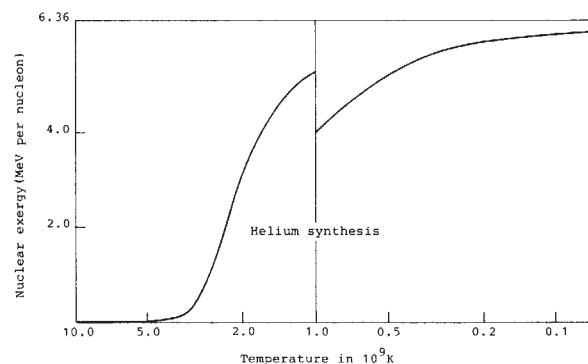
This equation differs from the equation (2) in the paper in that for each nuclide, T/A_i appears as an 'effective temperature', taking into account the reduced number of degrees of freedom of bound nucleons.

From the correct equation (2*) we obtain the correct expression for the exergy per nucleon after helium formation,

$$E(T) = \mu(T) - 2\gamma_0 b_3 - kT \ln \frac{2 \left[\kappa r \left(\frac{mc^2}{kT} \right)^{3/2} \right]^{1-\frac{3}{2}\gamma_0}}{(1-2\gamma_0)^{1-2\gamma_0} \gamma_0^{\frac{1}{2}\gamma_0}} \quad (10^*)$$

with $\mu(T)$ approaching b_4 for small T then (11*) obtains.

Figure 2 of the paper has to be modified, and the corrected Fig. 2 (below) shows the creation of exergy as given by (9) and (10*).



A couple of important references should also be added. Davis' book¹ on time asymmetry should have been referred to in the original paper. Frautschi's later paper² on negentropy in the Universe is also relevant. The model has been discussed by van Hove³ in connection to other time-asymmetric processes of the early Universe.

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Late Palaeozoic Wrangellian palaeomagnetic directions north or south seeking?

In a recent *Nature* article Panuska and Stone¹ report that they have resolved the polarity ambiguity in the late Palaeozoic palaeomagnetic data for Wrangellia. They have measured magnetic directions from the Lower Permian Hasen Creek Formation and the Lower Permian-Pennsylvanian Station Creek Formation which they conclude would place Wrangellia north of the Equator in the late Palaeozoic. They reason that since the Earth's field was predominately reversed at this time, any directions measured from these rocks would point to the reversed polarity geomagnetic pole, that is, the geographic south pole. However, their interpretation may be at odds with their data. Most palaeomagnetic studies report palaeomagnetic data just as it was measured from the rocks. If the southwest and down directions (Table 1 in ref. 1) are what was measured in the late Palaeozoic Wrangellia rocks, then these reversed directions would suggest that Wrangellia was south of the Equator and not north of the Equator, as Panuska and Stone¹ conclude. Two statements in Panuska and Stone's¹ article suggest that the southwest and down directions reported in Table 1 are not those that were actually measured. Both occur on page 562. "Thus taking into account the reversed geomagnetic polarity, the VGPs corresponding to the mean vectors plotted in Fig. 2 represent an estimate of the location of the north geographical pole for that time."¹ and "The polarity of the tabulated data represent the equivalent of today's Northern Hemisphere pole."¹ Although these statements may imply that the data have been changed to correspond to the authors' polarity interpretation, nowhere do the authors clearly state what directions were actually measured.

The reason for this comment is to point out that it is misleading and confusing if the data are not reported exactly as measured. Interpretations should be made only after the reader has access to the raw data. In this particular case it is important to know if the southwest and down directions were actually measured in the rocks, in which case Panuska and Stone's¹ interpretation is incorrect, or if the opposite polarity were measured.

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PANUSKA AND STONE REPLY—We welcome the opportunity to clear up the misunderstanding of data that we reported in our 1981 paper. The directions of the north-seeking magnetic vectors that we measured in the Skolai Creek rocks are actually northeasterly and negative (up). Since only summary data (that is, mean directions) were reported in our Table 1, and not the raw data, we inverted the mean directions in the interest of saving space by avoiding the use of separate tables for measured directions and inferred directions and "avoiding confusion" by reporting the probable polarity. The two statements cited by Kodama were intended to indicate that the measured directions were inverted for this paper because of the predominant reversed geomagnetic polarity during deposition of the Skolai Creek rocks. We have apparently failed in our attempt to avoid confusion and we thank Dr Kodama for bringing this to our attention.

To summarize, we measured northeast and negative (up) magnetic directions in the Skolai Creek rocks. These directions were inverted to the southwest and positive (down) directions reported in Table 1 because of the reversed geomagnetic polarity during the Permo-Carboniferous. Thus, we stand by our original interpretation of a Northern Hemisphere location for Wrangellia in late Palaeozoic time.

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Noise and recognizability of coarse quantized images

WE are pleased that Morrone *et al.*¹ found it still worthwhile a decade later to elaborate on an experiment by Harmon and Julesz², even though they disagree with our conclusions. We comment only because: (1) from Morrone *et al.* the reader might assume that Harmon and Julesz had critical-band masking as their only explanation²; (2) they failed, so far, to disprove critical-band masking; and (3) their demonstration does not seem convincing. We comment now in detail along those lines.

(1) Harmon and Julesz² offered two possible explanations for the inability to see Abraham Lincoln's face in the block-quantized image. One was discussed by us at length, but not quoted by Morrone *et al.*¹, although they give a similar explanation. We assumed that the visual system was very sensitive to line contours at the boundaries of the quantized blocks and

that these contours masked the low-spatial-frequency image. Our alternative explanation was critical-band masking, since we showed that the removal of an annulus-shaped spectrum from the Fourier spectrum of the block-quantized image restored the visibility of Lincoln's face. (This annulus had an inner radius f_0 and outer radius $2f_0$, where f_0 is the highest frequency in the Lincoln image.) Thus the quantization noise of the middle-frequency annulus next to the image spectrum was adequate to mask the image. This restoration of recognition was the more remarkable since some of the line contours around the quantized blocks were still visible. We also demonstrated that by filtering out the high frequencies outside the disk with $2f_0$ radius, the image of Lincoln remained hidden. This filtering removed the sharp contours at the boundaries of the quantized blocks, yet the face remained masked. It was these findings that persuaded us to consider critical band masking as an alternative to the trivial first explanation.

(2) Critical-band masking occurs for one-dimensional gratings and noise as shown by Stromeyer and Julesz³. This was recently reconfirmed by Wilson *et al.*⁴. In another study, Phillips and Wilson⁵ showed that for 2.5–6 cycles per deg test and masking gratings, the orientational tuning curves (half-amplitude bandwidths) are as shallow as 25 arc deg. While Morrone *et al.* did not specify the viewing distances in their experiment, one can assume that much of their 'windmill' noise falls in the above mentioned spatial-frequency range. It is not surprising that the windmill noise only 22.5° away from the axes of the two-dimensional Fourier domain will mask the masking noise; this is where the quantization-noise spectra are concentrated. Such a masking of the masker (disinhibition) was reported by Stromeyer and Julesz³.

According to our comment, we would like to see a demonstration by Morrone *et al.* with windmill noise restricted to the diagonals (say, between 40 and 50 arc deg.). Only if this noise were to unmask the original image would we regard the idea of critical-band masking as being challenged. Even then many questions would still be left unsettled. For instance, we used concentric filters with circular symmetry that yielded the same 1-octave-wide critical band for two-dimensional stimuli that were found by Blakemore and Campbell⁶ for one-dimensional stimuli. Whether asymmetric filtering can be used without causing some nonlinear effects remains to be seen. The finding by Daugman⁷ and Phillips and Wilson⁵ that two-dimensional spatial frequency channels are inseparable in their polar coordinates should caution us.

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(3) In Fig. 1d of Morrone *et al.*¹, the Mona Lisa is so poorly perceived that it resembles our Fig. 3a in ref. 2, which we called a masked image. We wonder how Morrone *et al.* can regard the image in their Fig. 1d "of higher quality than the lowpass filtered version". Since they did not use some objective method to compare the visibility of the *same* images masked by their windmill with our annulus-shaped spectral noise, any inference to critical-band masking appears superficial.

Finally, consider the more general problem that Morrone *et al.* indirectly questioned: the validity of spatial-frequency channels in vision. If critical band masking does not take place in two-dimensional vision, then spatial-frequency-tuned channels have a limited role. That in preattentive vision only local line contours can be detected, and global periodicities are unnoticed, led one of us to a texton theory of texture discrimination and effortless perception⁸⁻¹⁰. However, in attentive vision such as looking for a recognizable face, global processes (including the detection of periodicities) must be important. Whether the detection of these periodicities is undertaken by early analysers (which can be fatigued or masked) or is the result of higher cognitive processes (for which critical-band masking is not expected) remains to be seen.

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MORRONE *ET AL.* REPLY—For us the most intriguing aspect of Harmon and Julesz's¹ ingenious demonstration is that blocking does not merely degrade the image, but renders recognition impossible. Normal masking (such as their Fig. 3a¹ or indeed our Fig. 1d²) degrades the image but leaves it still recognizable.

Measurements in our laboratory show that masking with one-dimensional noise at 22.5° reduces threshold of high spatial frequency gratings by a factor of only 2,

compared with a factor of more than 30 for parallel noise³. In any event, even if the noise did encroach into the critical band of the spurious high frequencies it would add much more energy than it could have removed by masking.

Finally, both we² and Piotrowski and Campbell⁴ have demonstrated that recognizability is restored by phase scrambling of the spurious high frequencies, without interfering with their amplitudes, either physically or by masking. This is interesting, as classical masking and summation are phase independent (ref. 5 and M.C.M., D.C.B. and J.R., unpublished).

We do not challenge the notion of critical-band masking. We recognize the importance which it clearly has in visual processes, but we point out that it is not a sufficient explanation here. There is a substantial body of evidence, both from single cell recordings in cat and of evoked potentials in man, for nonlinear inhibitory processes. These processes presumably have some role in visual analysis, which we attempted to elucidate.

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Identification of γ -ray lines observed from SS433

RECENTLY, Lamb *et al.*¹ reported preliminary evidence for γ -ray line emission from the region of SS433. These authors suggested that observed γ -ray line features at 1.2 and 1.5 MeV may be the red- and blue-shifted components of the 1.369-MeV line from the first excited state to ground-state transition in ²⁴Mg. Here we point out some serious difficulties with this interpretation of the observed γ -ray lines.

In their model, Lamb *et al.*¹ propose that fast-moving ²⁴Mg nuclei ($v \approx 0.26c$, that is, 33 MeV per nucleon) undergo inelastic collisions with ambient protons to produce the 1.369-MeV γ ray. The apparent absence of similar inelastic γ -ray lines from normally more abundant isotopes such as ¹²C and ¹⁶O is interpreted as evidence for the absence of these nuclei in SS433. Although such an elemental distribution would be very different from that observed in the Solar System, such a possibility cannot be ruled out on this basis

alone. However, there are ways to test this hypothesis.

γ -Ray production cross-sections have been measured by Dyer *et al.*² for proton-induced reactions on C, N, O, Ne, Mg, Si and Fe from threshold to $E_p = 23$ MeV. For proton bombardments of ²⁴Mg at $E_p = 23$ MeV, in addition to the 1.369-MeV line, there is a strong doublet near 1.64 MeV from the 1.634-MeV ²⁰Ne line produced via the ²⁴Mg(p, p α)²⁰Ne reaction and the 1.636-MeV ²³Na line produced via the ²⁴Mg(p, 2p)²³Na reaction. At this bombarding energy, the ratio of the cross-sections for the 1.64-MeV line and the 1.369-MeV line is 0.85. Zobel *et al.*³ report a ratio of ~ 0.9 at $E_p = 30$ MeV. Thus, if the 1.2- and 1.5-MeV features observed by Lamb *et al.*¹ were due to inelastic scattering of ²⁴Mg on protons, one would expect to observe lines of nearly the same intensity at ~ 1.4 and 1.8 MeV.

The apparent absence of any significant γ -ray lines other than those at 1.2 and 1.5 MeV thus argues strongly against the proposed model of Lamb *et al.*¹. It is also interesting to note that the presence of ²⁴Mg lines and the absence of other lines not only would make Mg overabundant compared with carbon and oxygen, but would suggest that it is overabundant compared with heavier elements, particularly Fe, in view of the large cross-sections found at $E_p = 23$ MeV for the production of γ rays from Fe at 0.847, 0.931 and 1.317 MeV². Thus, it seems that an alternative explanation must be sought for the intriguing observations made by Lamb *et al.*¹.

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